PLANTS

Fundamental and mission-oriented research is supported which provides basic understanding of mechanisms and processes of plant systems. This knowledge is necessary as a basis for developing management strategies for improving and sustaining agricultural production systems. Grants are awarded under five programs: Plant Genome, Plant Genetic Mechanisms, Plant Growth and Development, Photosynthesis and Respiration, and Nitrogen Fixation and Nitrogen Metabolism.

PLANT GENOME

Panel Manager - Dr. Richard Michelmore, University of California - Davis
Program Director - Dr. Ed Kaleikau

This competitive grant program is part of the USDA Plant Genome Research Program. The Plant Genome Research Program was established in order to facilitate development of new or improved crop and forest species, thereby promoting sustainability and profitability of plant production and improvement of quality of food, fiber, and feed. To accomplish these goals, the program gives high priority to research for construction of genomic maps, and for detailed studies of specific regions of genomes, genes, or genetic processes. The program also supports research on development of new methods or innovative approaches that have potential application to create new germplasm/varieties, genome mapping, gene isolation or gene transfer in crop and forest species.

9701472 Systematic Reverse Genetics of the P450 Gene Family in *Arabidopsis*
Feldmann, K.A.; Feyereisen, R.; Galbraith, D.W. **Grant 97-35300-4864**

University of Arizona
Department of Plant Sciences & Department of Entomology **$330,000**
Tucson, AZ 85721 **3 Years**

This project concerns the functional analysis of cytochrome P450 enzymes in plants. These enzymes are encoded by genes in large gene families; estimates from current genome projects suggest more than 80 P450 genes in humans. Plants have even more, with a possible 160 in *Arabidopsis thaliana*. Common features of the sequences allow most P450 genes to be recognized, but finding functions for the enzymes encoded by the genes is very difficult.

It is well known that P450 enzymes are involved in many aspects of metabolism, including biosynthesis as well as detoxification. However, the methods available to elucidate these functions are slow. This project would provide new methods to rapidly assign function to different P450 genes. In brief, individual P450 genes are randomly "knocked-out" by insertional mutagenesis in *Arabidopsis*. Plants containing these knocked-out genes are individually identified by DNA amplification involving the polymerase chain reaction. Amplification is based on our knowledge of the gene sequences that distinguish P450s. We have also devised a simple pooling strategy to identify mutant plants with minimal effort; this in principle should allow us to find all 160 mutants in which the individual P450 genes are non-functional. These mutants will be fully characterized to find out which biochemical pathways have become blocked as a result of losing the specific P450 activity. The genes uncovered in this manner should profoundly impact plant biotechnology, particularly in terms of manipulating plant metabolism to produce desired compounds, or to resist environmental (biotic and abiotic) stresses.

9701402 Dissecting Genetic Control of Growth Rhythm in Douglas-fir through QTL Mapping
Neale, D.B.; Wheeler, N.C.; Ritchie, G.A. **Grant 97-35300-4623**

USDA Forest Service
Institute of Forest Genetics **$150,000**
Albany, CA 94701 **3 Years**

Douglas-fir is the most ecologically and economically important forest tree species of the Pacific Northwest. It has a broad and environmentally diverse range to which it is highly adapted to seasonal cycles and their attendant environmental signals. The annual growth rhythm, as determined by the timing of bud flush in the spring and bud set in mid-to-late summer, is precisely adapted to these environmental differences. Bud flush and bud set timing determines susceptibility to late spring or early fall frosts, respectively. The timing of spring bud flush is controlled primarily by two environmental stimuli; (1) chilling history during winter dormancy, and (2) temperature sum during quiescence. The timing of bud set is controlled by; (1) photoperiod, and (2) moisture stress. We are using QTL mapping as an experimental approach to determine the numbers of genes and their relative effects responding to different environmental stimuli in determining growth rhythms in Douglas-fir. The identification of QTLs for adaptive traits in Douglas-fir might ultimately be applied in marker-aided breeding for trees more precisely adapted to different environments.

9701779 Positional Cloning of *Triticum monococcum* Vernalization Genes
Dubcovsky, J. **Grant 97-35300-4379**

University of California, Davis
Department of Agronomy and Range Science
Davis, CA 95616-8515

New Investigator Award
$250,000
3 Years

The adaptability of bread wheat to a large range of environments is partially due to the exploitation of genetic differences in sensitivity to vernalization. The objective of this project is to develop the molecular tools required for the positional cloning of these important genes. Cloning
of wheat vernalization genes will not only provide a new understanding of the biochemical nature and genetic regulation of the vernalization response in winter cereals but also provide a new tool for the manipulation of the vernalization response in wheat.

The first objective of this proposal is to develop high-resolution genetic maps of vernalization genes in wheat. Two crosses between spring and winter diploid wheats will be used to generate large mapping populations. Available molecular markers for the targeted chromosome regions will be mapped simultaneously with the vernalization response. High throughput molecular marker technologies and regional targeting strategies will be used to develop closely linked markers encompassing the vernalization genes.

The second objective of this proposal is to clone the complete genome of diploid wheat using Bacterial Artificial Chromosomes (BACs). The BAC library will have one hundred BAC clones with inserts of 150 to 200 kilobases and will be a long-term tool for positional cloning of different
cereal genes.

The most closely linked markers flanking each vernalization gene will be used to screen the BAC library. The ends of the selected BAC clones will be mapped in the high-resolution genetic maps to confirm the presence of the genes within the selected BAC clones.

9701470 Marker-Assisted Introgression of Blackmold Resistance Genes into Tomato
St. Clair, D.A.; West, M.A.L. **Grant 97-35300-4621**

University of California, Davis
Department of Vegetable Crops
Davis, CA 95616-8746

$150,000
2 Years

The fungus *Alternaria alternata* causes Blackmold disease on ripe tomato fruit. Blackmold is a major cause of crop loss in processing tomatoes and decreased tomato product quality for consumers. The fungicides currently being utilized to control this disease are being eliminated due to environmental and consumer safety concerns. No documented Blackmold resistance exists in cultivated tomato (*Lycopersicon esculentum*). Five genes governing resistance to Blackmold are being transferred from a wild tomato relative (*Lycopersicon cheesmanii*) to cultivated tomato using DNA marker-assisted selection. Our laboratory had previously mapped the five resistance genes to the tomato chromosomes using DNA markers. We are currently developing a new DNA marker technology to trace these resistance genes during transfer from wild to cultivated tomato (in order to select those tomato lines that contain the desired resistance genes). Breeding lines with different combinations of the five resistance genes will be used to examine the effects of each gene on the expression of resistance. Cultivated tomato breeding lines with enhanced resistance to Blackmold and the DNA markers associated with the resistance genes will be released publicly for further breeding efforts.

9701934 Genetic Definition of Host Resistance Against Parasitic Weeds
Yoder, J.I. **Grant 97-35300-4622**

University of California, Davis
Department of Vegetable Crops
Davis, CA 95616-8746

$100,000
2 Years

Parasitic weeds are enormously destructive agricultural pests. For example, dwarf mistletoes are the most widespread and damaging pathogens in commercial forestry in the western United States. The unrelated parasitic plant dodder is also widespread in U.S. agriculture and causes significant yield losses to grain and vegetable crops. While host resistance is generally considered a key component of integrated pest management strategies, efforts to develop effective resistances against parasitic weeds have made little headway. The long-range goal of this work is to engineer resistance to parasitic weeds in crop forestry plants.

For several reasons it is difficult to study the genetics of parasitic weed - host interactions. Therefore, we are using a model parasite - host system to identify genes essential for parasitism. Two plant species favored for genetic studies, *Arabidopsis* and tomato, are efficiently parasitized by the root parasite *Triphysaria*, a member of the *Scrophulariaceae* family closely related to *Striga* and *Orobanche*. Because both the hosts and the parasite are amenable to genetic analysis, we think this is an excellent system for identifying genes that might be useful to develop resistant crops. We are screening *Arabidopsis* and tomato populations for individuals incapable of supporting *Triphysaria*. The screen we are using will uncover genes critical at different stages in the

development of parasitism. Once identified, the gene will be cloned and reintroduced into crops most susceptible to parasitic weeds. This will offer an unprecedented level of protection against a serious and ubiquitous agricultural pest.

9701801 Application of DNA Microarrays to Problems in Plant Pathology
Somerville, S.C. **Grant 97-35300-4863**

Carnegie Institution of Washington
Department of Plant Biology **$165,000**
Stanford, CA 94305-1297 **2 Years**

New technologies will be required to exploit the vast resources of cDNA and genome sequence information. One such technology is the DNA microarray, developed by colleagues at
Stanford University. The mRNA abundance of up to 10,000 genes can be assayed with one microarray, representing a ~1000-fold increase in the number of genes that can be analyzed at one time.

Like many technical advances, the DNA microarrays make possible experiments that were previously impossible. Specifically, global analysis of the expression of most or all genes of an organism can be performed giving a more holistic view of responses to environmental or developmental cues. In agriculture, this technology will expand our understanding of how plants integrate responses to various environmental stresses (e.g., drought stress, nutrient limitations, toxic soils). In addition, it will bring a greater understanding of fundamental processes that have been difficult to study due to their inherent complexity (e.g., the allocation of resources between growth and seed, flowering timing, plant architecture).

Our primary goal is to adapt this new technology for use with plants, and the first objective outlines several optimization experiments. As a demonstration, the mRNA levels of 500 genes will be compared in resistant and susceptible plants to provide the first global view of how plants respond to pathogen attack. Also, the microarrays will be used to survey a large collection of powdery mildew resistant lines to identify plant lines with novel resistance mechanisms.

9701389 Parallel Computing for Physical Mapping of Fungal Genomes
Bhandarkar, S.M.; Arnold, J. **Grant 97-35300-4647**

University of Georgia
Department of Computer Science **$200,000**
Athens, GA 30602-7404 **3 Years**

The problem of reconstructing a physical map of an entire fungal or plant genome from a random collection of DNA fragments (or genome library) presents a central computational and statistical problem in genetics and provides fundamental insights into fungal and plant development, gene organization, chromosome structure, recombination, and the role of sex in evolution. This fundamental reconstruction problem is equivalent to a very famous problem in computer science i.e., the Traveling Salesman Problem, in which a salesman must trace a route through a list of cities in such a way as to travel the shortest distance. This problem, and hence the physical mapping problem, is very hard to solve. We propose to develop new algorithms that exploit parallel computing i.e., utilize several computers simultaneously, to solve this problem. Parallel computing can lead to substantial increases in speed required for physical map reconstruction and for assessing its reliability, especially for larger and more complex plant genomes. The proposed research addresses the long-term goal of the USDA Plant Genome Program of developing novel technologies for genome mapping, genome manipulation, gene isolation and gene transfer in plants. These new parallel algorithms for physical mapping will be applied to the model fungal systems *Aspergillus nidulans*, *Aspergillus flavus* and *Neurospora crassa*, and the peanut genome. The project will play a pivotal role in the identification and manipulation of genes involved in aflatoxin production in peanuts, identification of signal transduction pathways for aflatoxin production, the identification of new targets for antifungal agents transformed into peanuts, and the identification of differences between toxigenic and atoxigenic fungal strains for the purposes of biocontrol.

9701471 Identifying Heterotic Regions in the Tetraploid Alfalfa Genome
Brummer, E.C. **Grant 97-35300-4573**

Iowa State University
Department of Agronomy **$150,000**
Ames, IA 50011-1010 **3 Years**

Alfalfa (*Medicago sativa L.*) is the most economically important forage crop in the United States, forming the basis of the dairy and hay industries as well as significantly contributing to all other livestock enterprises. Improved cultivars are necessary for these systems to remain profitable. Current methods of developing cultivars could be enhanced with better knowledge of alfalfa genetics. Alfalfa is normally cross-pollinated; self-fertilization results in inbreeding depression, manifested as severe loss of forage and seed yield. Progeny from intercrossed, unrelated plants exhibit heterosis (or hybrid vigor), the opposite of inbreeding depression. The goal of this project is to identify regions in the alfalfa genome that control heterosis/inbreeding depression. Molecular markers will be used to develop a comprehensive genetic map of the tetraploid alfalfa genome in two populations

derived from the same parents. One population will be non-inbred and express heterosis; the other will be inbred for one generation. Forage and seed yield will be determined in field and greenhouse trials for two years. Markers associated with forage and seed yield heterosis or inbreeding depression will be identified. Plant breeders can use these markers to select plants with desirable, and complementary, blocks of genes that will maximize heterosis. Introducing favorable genes or alleles from wild germplasm and developing populations of alfalfa that express heterosis when intercrossed will be facilitated by identification of these chromosomal regions. This project will provide a better understanding of the genetic control of heterosis in crop plants.

9701723 Fine-Mapping a Crown Rust Resistance Gene Cluster in Diploid Oat
Holland, J.B. **Grant 97-35300-4865**

Iowa State University **New Investigator Award**
Department of Agronomy **$115,000**
Ames, IA 50011-1010 **3 Years**

Crown rust disease is the most damaging disease of oats in the U.S., causing significant losses in grain yield and quality every year. Genetic resistance to crown rust is the most economical and environmentally sound method of protection against the disease. The primary sources of genes for resistance to crown rust are the wild relative species of oats. We have identified a cluster of crown rust resistance genes all located in the same chromosomal region of a wild oat species, *Avena strigosa*. We propose to genetically "dissect" this complex group of genes using classical genetic techniques of crossing lines possessing different portions of this cluster together and examining their progeny for lines that contain only single resistance genes. This will allow us to unequivocally identify the individual genes that make up this cluster. We will combine these classical genetic approaches with modern molecular genetic techniques to develop a detailed understanding of these resistance genes. First, we propose to complete a genetic map of the entire genome of this species using DNA markers. In addition, we propose to identify numerous DNA markers within and surrounding the chromosomal region of this resistance gene cluster. The results of this research should provide information and materials (such as genetic stocks and DNA markers) that can be used by oat breeders to introduce and track these resistance genes from the wild species into cultivated oats, and lead to progress toward cloning of these resistance genes.

9701800 Genetic Stocks and Map Development for Improved Resolution of the Maize Genetic Map
Lee, M. **Grant 97-35300-4939**

Iowa State University
Department of Agronomy **$180,000**
Ames, IA 50011-1010 **2 Years**

Knowledge of the genetic control of traits will become a more important source of increased or sustained crop productivity because other inputs (e.g. fertilizer, pesticides, and irrigation) will become more expensive or have been exploited to their maximum utility. A common means of acquiring genetic knowledge about a trait is to determine the genetic and chromosomal positions of genes associated with the trait. Such information may be used to understand the biological basis of the trait, select and improve it, and clone important genes. Such information has been compiled into references for many types of genetic investigations. However, the amount of information has accumulated to the point where it has become very difficult to determine the genetic position of a gene with the commonly used corn genetic maps. The genetic stocks used to make those maps had minimal opportunities for recombination or crossing over. The corn map and genetic stocks developed with this project will allow researchers to resolve the order of genes along a chromosome with unprecedented power. The genetic stocks, recombinant inbred lines (RILs), were derived from a population which had been intermated to provide more recombination. The RILs will be fingerprinted with genes and DNA sequences from corn, rice, barley, sorghum and wheat so that information from those grain crops may be compiled and comprise a genetic resource for those crops. Also, the RILs are a permanent mapping population and they may be distributed so other laboratories and scientists may share information by virtue of working with a common mapping population.

9701465 Genetic Studies of Cereal Low Phytic Acid Mutants
Raboy, V. **Grant 97-35300-4421**

USDA Agricultural Research Service
National Small Grains Germplasm Research Facility **$150,000**
Aberdeen, ID 83210 **3 Years**

Grain crops contain enough total phosphorus to supply a large portion of a human or animal's nutritional requirements. However, about three-quarters of this phosphorus is in a chemical form, called phytic acid, that is excreted by humans and "non-ruminant" animals such as poultry, swine and fish. Phytic acid-derived animal waste phosphate contributes to water pollution, and excretion of phytic acid can contribute to mineral deficiencies in humans. A series of "low phytic acid" mutants of corn, barley and rice have been isolated. In these mutants, grain phytic acid phosphorus is reduced by about 50% to 75%, and "available" phosphorus (phosphorus that can be used by a human or animal) is increased by 50% to 75%. "Low phytic acid" crops

should be of value both as animal feeds and in human foods. Their use should help to reduce the environmental impact of agricultural production, and contribute to improvements in the efficiency, profitability and competitiveness of U.S. agricultural production. In this project, the number of different genes identified in these mutant collections will be determined. These genes will be "mapped" to pinpoint their chromosomal location. There will also be additional screening for mutants representing novel genes. A gene previously thought to be important to phytic acid synthesis will be cloned, and studies will be conducted to determine if any of the above mutants are mutations of this gene. This work will greatly support and extend the use of these mutants in the breeding and development of "low phytic acid" grain crops.

9701721 Parallel Studies of Genome Micro-Organization and Dynamics in Maize, Sorghum and Rice
Bennetzen, J.L. **Grant 97-35300-4594**

Purdue University
Department of Biological Sciences **$370,000**
West Lafayette, IN 47907-1392 **3 Years**

Although many researchers have studied the structure and expression of genes, few investigations have targeted the DNA sequences that reside between genes. In most higher organisms, both plants and animals, the majority of the DNA sequences in the chromosomes are not genes, and both the nature and roles of this non-genic DNA remain largely unknown. We will investigate the structure, function and evolution of the DNA sequences between genes in three grasses: corn, sorghum and rice. These species were chosen because they are important model species, because they are major crop plants, because they vary by over 5-fold in genome size (but no more than 2-fold in gene number), and because they represent very different times of evolutionary separation (about 15 million years for sorghum from corn and at least 50 million years for the ancestor of corn/sorghum from the ancestor of rice). A pair of comparable chromosomal regions were chosen from each of these species, so we can also investigate the evolution of intergenic DNAs. We will determine how DNA sequences are arranged in these two regions and how these arrangements have changed since the divergence of these three species. In addition, we will investigate the similarities and differences in chromosome folding, recombination, and gene expression in these two regions. The results of these experiments will allow us to develop new technologies for position-based cloning of plant genes and to understand how natural evolutionary processes have created the diversity of function present in these different grasses.

9701974 Molecular Markers for Resistance and Construction of a BAC Library in Strawberry
Haymes, K. **Grant 97-35300-4586**

USDA Agricultural Research Service **Postdoctoral Fellowship**
Fruit Laboratory **$90,000**
Beltsville, MD 20705 **2 Years**

The goal of this research project is to develop DNA markers for three resistance genes in the commercial strawberry that confer resistance against a fungal disease called *Phytophthora fragariae*. Previously, we utilized a technique called bulked segregant analysis to identify random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers to find linkage to *P. fragariae* resistance genes. The bulked DNAs represented subsets of three F1 populations that segregated monogenetically (1:1) for either resistance or susceptibility to *P. fragariae*. A genetic map was constructed and the markers linked to the resistance gene were shown to be linked. Based upon these initial results, we propose the following objectives: 1) to convert the genetic markers linked to the genes into highly specific markers, 2) to assess the strawberry germplasm for the conservation of the molecular markers to the genes, and 3) to construct a bacterial artificial chromosome library of the strawberry so that we may someday obtain the actual genes for resistance to this disease.

Strawberry breeding programs will benefit from the application of these molecular markers linked to resistance genes by providing an efficient means to screen and select plant material containing resistance genes, and by allowing marker-facilitated selection of these resistance genes. A long-term goal of this research is to reduce the amount of chemical fumigants used to grow strawberries. Genetic improvement of the strawberry by the ability to pyramid resistance genes will contribute to the productivity of the strawberry industry.

9701667 Molecular Analysis of Maize Centromeres
Birchler, J.A. **Grant 97-35300-4380**

University of Missouri
Department of Biological Sciences **$350,000**
Columbia, MO 65211 **3 Years**

Centromeres are the regions of chromosomes that carry them through cell divisions. Previous work has shown that they consist of repeated units of DNA. The specific nucleotide sequence of these repeats and their higher order arrangement has not been determined. The proposed research will determine these aspects of maize centromeres. Sections of a centromere that are determined to be critical for function will be cloned in a vector that will accept large segments of DNA. Selected clones will be

introduced back into maize tissue culture cells to test their ability to function as an "artificial chromosome." An alternative approach to generate an artificial chromosome will involve the continued fracture of a pre-existing minichromosome to determine the minimum size that will retain transmission ability through the maize life cycle. The development of artificial chromosomes in plants has the potential for significant advances in agricultural biotechnology because they will permit the introduction of large fragments of DNA into plants or the introduction of genes for a complete biochemical pathway for a desired product.

9701571 Mutants and Biochemically Defined Loci Mapped in Concert
Coe, E.H.; McMullen, M.D.; Polacco, M.L. **Grant 97-35300-4383**

USDA Agricultural Research Service
Curtis Hall, University of Missouri **$200,000**
Columbia, MO 65211-7020 **3 Years**

Advances in genetics and molecular biology of the last 50 years provide a foundation on which a new era exists for understanding the genetic material of crop plants such as corn (maize), and the role of individual genes in complex traits. Mutational analysis, DNA characterization, molecular mapping, and quantitative genetics in combination provide opportunity for better understanding of factors contributing to normal plant development and of complex traits and crop improvement. In maize there are abundant mutants to exploit in furthering the quest to identify the role each gene plays in the developmental program. Some questions to which this project is directed are (1) How easily can associations be recognized between visible mutants and identified DNA, or other biochemically defined genes? (2) How often can potential bedfellows be recognized between visible mutants and quantitative trait genes, by coincidence of their map positions in the chromosomes? (3) What proportion of genes in the genome can be identified by their visible expression as simple mendelizing variants; what proportion have no visible consequence in the plant or seed? Associating of DNAs with visible expressions, and expressions with quantitative (i.e., measured) traits such as yield, kernel quality, and plant strength or resistances would be of highest economic importance. The project contributes to construction of high density, integrated chromosome maps specifically for maize and, by common genetic sequences (synteny), for other cereal grains, and to development of efficient genetic designs and mapping methods applicable to crops in general.

9701357 Genetic Control of Corn Earworm Resistance Factors in Maize
McMullen, M.D.; Lee, E.A.; Byrne, P.F.; Snook, M.E.; Wiseman, B.R.; Widstrom, N.W.
Grant 97-35300-4391

USDA, ARS, Plant Genetics Research Unit
Curtis Hall, University of Missouri **$364,000**
Columbia, MO 65211 **3 Years**

Recent advances in molecular marker technology have provided tools for determining the genetic basis of complex agronomic traits for major crop species. However, the interpretation of these quantitative trait locus (QTL) studies is limited by the lack of information on the metabolic pathways leading to most economic traits. Inferences about the roles of the underlying genes with a biochemical pathway or the nature of their interaction with other loci are generally not possible. An exception is resistance to the corn earworm (CEW) in maize due to the chemical compound maysin, a C-glycosyl flavone, synthesized in silks via a branch of the well-characterized flavonoid metabolic pathway. Our results using maysin synthesis as a model QTL system demonstrate the importance of regulatory genes and the importance of genes in interconnecting biochemical pathways on QTL expression. In this project we will continue our combined QTL/pathway candidate gene to address additional specific steps or mechanistic concepts for flavonoid synthesis. Improved understanding of the genetic basis of maysin synthesis and associated CEW resistance should lead to novel breeding strategies. More broadly, the insights gained in relating a defined genetic and biochemical pathway to a quantitative trait should enhance interpretation of the biological basis of variation in other quantitative traits.

9701350 1997 Gordon Research Conference on Plant Cell Genetics and Development
Messing, J. **Grant 97-35300-4214**

Rutgers, The State University of New Jersey
Waksman Institute **$5,000**
Piscataway, NJ 08855-0759 **1 Year**

The Gordon Research Conference on Plant Cell Genetics and Development (PCG&D) is a conference now held in alternate years with the Plant Molecular Biology conference. The last PCG&D conference was held in June 1995 and the program emphasized studies of apical meristem and primordia. Last year's Plant Molecular Biology conference in July 1996, included sessions on gene silencing, light signaling, plant hormone action, plant disease resistance, etc. The upcoming PCG&D conference will be held in June 1997 at New England College in Henniker, New Hampshire. Although the program will cover a broad range of subjects on plant genetics, it will highlight the importance of synteny of plant genomes on our understanding of gene architecture and function.

9701677 Testing Novel Methods for Sequence Analysis of the Maize Genome
McCombie, W.R.; Martienssen, R.A. **Grant 97-35300-4564**

Cold Spring Harbor Laboratory **$370,000**
Cold Spring Harbor, NY 11724 **3 Years**

The genome of maize, as well as of most other crop plants, is extremely large and comprised mostly of a large number of copies of a few repeat sequences. This structure greatly complicates and increases the cost of any effort to analyze the genome of this important crop. We will test several methods to use the properties of the repeats to remove these sequences from samples of maize DNA being analyzed. This will enable us to carry out DNA sequencing and other experiments using a portion of the maize genome enriched for genes, which are the most interesting portion of the genome from a commercial standpoint. There is an international effort underway to sequence one of the smallest genomes of a flowering plant, that of *Arabidopsis*. The small size of this genome (about 1/20th the size of maize) makes it an extremely efficient plant to study. The information gained from the studies we are beginning here may allow us to use the sequence of *Arabidopsis* in even more ways than anticipated to find and analyze maize genes. This will increase the benefit of the investment in *Arabidopsis* and allow maize research to proceed in a more cost effective manner. We will also isolate and sequence a large number of random genes in the form of cDNA tags or ESTs, as they are sometimes known. We will make all of these sequences available to the research community by immediately releasing them through our World Wide Web site.

9701792 Development of a BAC library for apple and cloning of the *Vf* gene
Weeden N.F.; Brown, S.K.; Aldwinckle, H.S. **Grant 97-35300-4587**

Cornell University
Department of Horticultural Sciences **$150,000**
Geneva, NY, 14456 **3 Years**

Apple is the most important deciduous tree fruit crop in the United States. The major fungal disease affecting apple in North America and in most other temperate regions is apple scab, and its control may require ten to twelve pesticide sprays per year. Fortunately genes conferring resistance to scab exist in wild apple germplasm, and through a lengthy breeding process can be incorporated into commercial breeding stock. However, the resistance genes cannot be introduced into varieties currently available without the use of biotechnology. In this project we intend to develop one of the biotechnological tools necessary for isolating genes from apple, a bacterial artificial chromosome (BAC) library, and use this tool to isolate the most important gene (*Vf*) responsible for scab resistance in apple. This gene can then be used to directly convert susceptible varieties into resistant ones without affecting other fruit or tree characteristics. A BAC library usually consists of between 20,000 and 40,000 individual DNA clones, each containing a relatively large piece of apple DNA so that the entire apple genome is represented in the collection. We will screen the apple library for the presence of DNA sequences known to lie very close to *Vf*, as well as for sequences known to have a structure similar to disease resistance genes isolated from other plant species. This two-pronged approach should permit the identification of those clones containing *Vf*. Confirmation of *Vf* will be by conversion of a susceptible variety into a resistant one using transformation procedures already available.

9701436 Cloning of a Recessive Bacterial Disease Resistance Gene from Rice
Blair, M.W. **Grant 97-35300-5101**

Cornell University **Postdoctoral Fellowship**
Department of Plant Breeding **$90,000**
Ithaca, NY 14853-1902 **2 Years**

Plant pathogens cause disease by producing virulence factors that manipulate host metabolism to the plant's detriment and the pathogen's benefit. These virulence factors may increase disease susceptibility by tricking plant proteins into becoming unwitting collaborators of the pathogen. However, the molecular mechanisms of disease susceptibility are not as well understood as the recognition and defense response systems that can be activated by plants when they are attacked. This research will determine whether resistance can result from recessive mutations that block disease susceptibility genes in plants. Recessively-inherited resistance is a common phenomenon for diseases caused by fungal, bacterial and especially viral plant pathogens. In this project, a recessive resistance gene from rice, xa-5, that provides vertical, race-specific resistance to the bacterial leaf blight pathogen, *Xanthomonas oryzae pv. oryzae*, will be isolated by a strategy of map-based cloning and complementation testing. Positional cloning has a successful track record in rice because of its small genome size and complete "toolbox" for genetic analysis. The cloning of the recessive resistance and dominant susceptibility alleles of xa-5 may uncover a unique class of genes and provide molecular evidence for how these genes function. The structure of the cloned gene may help to explain how plant-pathogenic xanthomonad bacteria are able to attack so many of the vegetable, fruit, cereal and fiber crops important to U.S. agriculture. This knowledge will have significant practical implications for long-lasting management of resistance and for engineering new disease resistance strategies by interfering with the underlying processes of plant pathogenesis.

9701552 Identification and Characterization and Map-Based Cloning of Major QTLs Affecting the Size and Shape of Fruit in Tomato and other Solanaceous Species
Tanksley, S.D. **Grant 97-35300-4384**

Cornell University
Department of Plant Breeding and Biometry **$270,000**
Ithaca, NY 14853 **3 Years**

Fruit size and shape are two major factors determining yield, quality and consumer acceptability for many crops. Both traits are quantitatively inherited; and, thus far, none of the genes controlling either of these traits have been cloned. During the course of this project, we will clone and characterize two QTLs (*fw2.2* and *ovate*). These loci are known to control a major portion of the genetic variation for size and shape of tomato fruit. All loci have been pinpointed on the tomato molecular linkage map and *fw2.2* has already been isolated on a YAC clone and four candidate cDNA clones identified. Once these loci are cloned, we will 1) determine the location(s) and timing of their expression; and 2) determine whether the same genes also control fruit size and shape in other domesticated species through comparative mapping. The proposed research will result in the first molecular insights into the control of fruit size and shape, and may lead to the future engineering of these traits in many crop plants. Moreover, strategies to be implemented in this project (including the development and use of a BAC genomic library of tomato) may pave the way for cloning QTLs in other plants.

9701521 Reverse Genetics in Plants: A Deletion-Detection Approach
Poethig, R.S. **Grant 97-35300-4599**

University of Pennsylvania
Biology Department **$66,000**
Philadelphia, PA 19104-6018 **1 Year**

The ability to generate plants or animals in which the function of cloned genes has been disrupted (reverse genetics) provides a way of testing the function of these genes, and makes it possible to genetically engineer organisms for a variety of applications. The most widely used reverse genetic approaches either require that a species be amenable to transformation or that it harbor a highly mutagenic transposable element system. (e.g. Mu transposons in maize). We propose to test a simple, alternative approach for generating loss-of-function mutations in cloned genes. Using PCR primers for sequences located at intervals in and around a gene of interest, it should be possible to detect deletions that alter the spacing of these primers; such deletions should produce a fragment that is detectably smaller than the fragment amplified from wild type DNA. This approach will be tested using several well-characterized fast neutron-induced deletions of the HY4 gene in *Arabidopsis*. We also propose to test whether a number of chemical mutagens that cause small deletions in animals have a similar effect in plants. If successful, this method will make it possible to pursue reverse genetics in any plant species that can be efficiently mutagenized with either fast neutrons, or another agent that induces small deletions.

9701555 Molecular Tagging of Genes Conferring Early Blight Resistance in Tomato
Foolad, M.R.; Christ, B.J.; Chen, F.Q. **Grant 97-35300-4685**

Pennsylvania State University
Department of Horticulture **$110,000**
University Park, PA 16802-4200 **2 Years**

Early blight, caused by the fungus *Alternaria solani*, is one of the most common and destructive diseases of tomato in areas of heavy dew, frequent rainfall, and high relative humidity. In the United States, the disease can be very severe in the Midwest, eastern (including Florida) and northeastern regions. In Pennsylvania, for example, approx. 20% of the tomato crop is lost annually from the incidence of early blight. At present, sanitation, long crop rotation, and routine application of fungicides are the most common disease control measures. Genetic resistance provides the best strategy for durable control and is an environmentally sound approach. No sufficient resistance to *A. solani* is known in the cultivated tomato, *Lycopersicon esculentum*. However, sources of genetic resistance have been identified within *L. hirsutum*, a related wild species of tomato. This study was designed to discern the genetic basis of early blight resistance in tomato and to identify DNA markers that are linked to resistance genes and which can be used as indirect selection criteria in breeding programs. Segregating backcross populations (BC1 and BC2) of an interspecific cross between *L. esculentum* (disease susceptible) and *L. hirsutum* (disease resistant) will be used to identify genes (quantitative trait loci, QTLs) associated with early blight resistance. These populations will be evaluated for resistance under different conditions (field, greenhouse, and laboratory) and in two locations (PA and OH). Two approaches, marker-based analysis and trait-based analysis, will be used to identify QTLs. The stability of QTLs across generations, environments and experimental and biological conditions will be determined. The combined results will provide a comprehensive knowledge of the genetic control of resistance to *A. solani* and facilitate opportunities for rapid development of early blight resistance in tomato through marker-assisted selection. The results will also provide impetus for map-based cloning and characterization of resistance genes and for engineering resistant cultivars through genetic transformation.

9701388 Map-based Cloning and Transposon Tagging of Jointless-2 from Tomato
Wing, R.A. **Grant 97-35300-4678**

Clemson University
Department of Agronomy
Clemson, SC 29634-0359
$154,000
3 Years

Abscission is an extremely important and dynamic process whereby plants shed their organs. Although the process of separation between cells of fully developed abscission zones (AZ) has been well investigated, the process by which certain cells differentiate as an abscission zone is not well understood. The long-term goal of our program is to establish a model system that can be utilized to study the gene regulation and cell biology of abscission zone development. The immediate objectives of this 3-year proposal are: 1) First we will continue to use a map-based cloning strategy to isolate and characterize a YAC/BAC contig that contains the wild type *jointless-2* locus. Once a contig is obtained we will perform complementation tests by transforming *j-2* tomato lines with overlapping genomic subcones and candidate cDNAs derived from the contig. 2) If the *j-2* gene is cloned within the time frame of this proposal, it will be characterized at all possible levels to determine its molecular role in the development of floral abscission zones. For plant biology, the cloning of the *j-2* genes will provide a unique opportunity to study the molecular biology of AZ development. For agriculture, we may be able to transfer the jointless system into other crop plants. In essence, we want to give breeders a new tool which can function as an aid to mechanical harvesting. The potential seems greatest for indeterminately flowering species that exhibit natural flower and young fruit abscission, such as cotton, soybeans, tomatoes, and peppers.

9701466 Development and Utilization of Microsatellite Markers in Cotton
Reddy, A.S.; Kamal, E.Z. **Grant 97-35300-4585**

Texas A&M University
Department of Soil and Crop Sciences
College Station, TX 77843
$90,000
2 Years

Cultivated cotton is the fourth most valuable crop in the U.S. with aggregate value of $6.0 billion a year. The future of U.S. cotton production depends on continued genetic improvement and development of new superior cultivars. Strategies such as application of polymorphic DNA markers to breeding programs and introduction of novel genes into elite lines are needed to accelerate the development of new cultivars. Polymorphic DNA markers have numerous applications in the breeding of cotton and other crop plants, e.g., mapping and isolating genes controlling agronomically important traits, marker-assisted selection, germplasm screening, pedigree analysis and cultivar identifications. Several DNA marker systems have been studied in cotton except microsatellite markers. The level of polymorphism at microsatellite loci in plants is high relative to that of other types of DNA markers. The long-term goals of the research of which this proposal is a part are to identify DNA markers that can be utilized in public cotton breeding programs as diagnostic markers both for genotyping and marker-assisted selection of agronomically important genes. The specific intentions of this proposal are to develop 150 public-domain microsatellite loci and map these loci in cotton. These data will be integrated into the existing cotton genetic linkage map. We will also develop and use 10 highly polymorphic microsatellites for cotton germplasm analyses. The information generated in this project can be used for genotyping U.S. cotton germplasm collection and in screening agronomically important traits.

9701462 Toward a Unified Map of Cotton and Arabidopsis
Paterson, A.H. **Grant 97-35300-4623**

Texas A&M University
Department of Soil and Crop Sciences
College Station, TX 77843
$250,000
3 Years

The goal of this research is to utilize information about the genomes of facile models such as *Arabidopsis*, in the molecular dissection of large crop genomes such as that of *Gossypium* (cotton). The experimental approach proposed is based upon our discovery that many chromosome segments have retained similar gene order since the divergence of narrow-leaf (monocot) and broad-leaf (dicot) plants from a common ancestor about 130-200 million years ago. This approach offers particular benefits in molecular dissection of cotton, worth $4-6 billion/yr in the USA, and which lacks a small-genome relative that can be used to expedite "chromosome walking/landing."

"Unifying" the molecular maps of higher plant chromosomes will open new doors to analyzing similarities in the sets of genes that confer high productivity to different crops, and afford many economies in molecular dissection of crop genomes. Our results will expedite many aspects of
cotton genome analysis, particularly the identification of candidate genes that may control economically-important traits such as fiber quality.

This work will contribute directly to long-range improvement in, and sustainability of, U.S. agriculture, by (1) providing a model for the direct utilization of *Arabidopsis* genome information in molecular analysis of major crops, such as cotton, (2)

creating new tools and information needed to provide genetic solutions to future problems in U.S. cotton production, processing, and utilization, (3) setting the stage for positional cloning of developmental mutations unique to cotton, and (4) training of several young scientists in new approaches to crop improvement.

9701942 AB-QTL Breeding of Peanut: Merging Gene Introgression with Comparative Mapping
Simpson, C.E.; Starr, J.L.; Paterson, A.H. **Grant 97-35300-4584**

Texas A&M University, Stephenville **$123,000**
Stephenville, TX 76401 **3 Years**

We will simultaneously make the first comprehensive genome map of cultivated peanut, transfer valuable new genes into cultivated peanut from its wild relatives, and identify genes fundamental to domestication of leguminous crops. Transfer of new genes into peanut will reduce both genetic and environmental vulnerability of U.S. peanut production, valued at about $1 billion per year, and also contribute to improvement of other legumes. Mapping of a highly-polymorphic peanut population will fill a void in the U.S. agricultural research infrastructure, creating tools needed for future endeavors such as isolation of specific peanut genes or molecular dissection of variation among peanut genotypes in eliciting allergenic responses. The synthetic tetraploid TxAG6 provides many valuable new traits needed in the genetically-impoverished gene pool of cultivated peanut. The cultivated peanut will be integrated with the existing diploid map, and with maps of soybean, common bean, and mungbean. We will also map highly-conserved *Arabidopsis* cDNAs that are being used to unify the genetic maps of monocot and dicot taxa, extending this unification to the legumes for the first time. This work will contribute directly to long-range improvement in and sustainability of the U.S. food production system by expediting intrinsic genetic solutions to needs of agricultural producers, processors, and consumers. Finally, we will train several young scientists in contemporary, interdisciplinary approaches to improving productivity and quality of a crop worth more than $1 billion per year to the U.S. economy.

9701248 *Fusarium* Resistant Germplasm by Altered Gene Expression in the Barley Seed Coat
von Wettstein, D.; Kleinhofs, A. **Grant 97-35300-4598**

Washington State University
Departments of Crop and Soil Science & Genetics and Cell Biology **$280,000**
Pullman, WA 99164-6420 **3 Years**

Fusarium head blight is a serious disease of barley and wheat caused by several species of this fungus. It produces mycotoxins which kill animals and humans, if they consume infected grain. Therefore, all grain is screened for the presence of these mycotoxins and portions containing them are discarded. Epidemics of head blight in the Midwestern States have caused grain losses amounting to about $2-$3 billion during 1993-1996. Dihydroflavonols and other plant metabolites in the flavonoid pathway, which *inter alia* synthesizes the pretty flower pigments of roses and bluebells, inhibit the growth and spore formation of *Fusarium* species. Such fungitoxic flavonoid intermediates can be accumulated in the seed coat of barley by blocking the pathway at appropriate positions with induced mutations and thereby prevent its penetration by the fungal hyphae. However, the mutations block the pathway also in other tissues of the plant, where the end-product is needed for protection against sun irradiation and other environmental stresses. Germplasms with such mutants are, therefore, disappointing, since they lead to yield depressions and other agronomical defects. The lessen learned is: Natural fungicides have to be expressed tissue specifically. The present project endeavors to identify genes that are only expressed in the seed coat and to use their promoters for production of antifungal flavonoids in the seed coat by antisense gene technology.

PLANT GENETIC MECHANISMS

Panel Manager - Dr. Harry J. Klee, University of Florida
Program Director - Dr. Liang-Shiou Lin

This program area supports studies addressing the basic cellular, molecular and genetic processes which contribute new information required for the development of novel approaches to crop and forest improvement. Innovative research is emphasized in the following areas: (1) characterization of agriculturally important genes and gene products, (2) relationship between gene structure and function, (3) regulatory mechanisms of gene expression, (4) interactions between nuclear and organellar genomes, (5) mechanisms of recombination, transposition, replication, and repair (6) genetic mechanisms affecting diversity in natural and crop/forest plant populations.

9701598 Tobacco Chloroplast DNA Replication Origins and Origin-binding Proteins
Nielsen, B.L.; Singh, N.K. **Grant 95-37301-2079**

Auburn University
Department of Botany and Microbiology **$70,000**
Auburn, AL 36849-5407 **2 Years**

While much is known about chloroplast gene expression, until recently little has been reported on the mechanism of DNA replication in this photosynthetic organelle. It is known that chloroplast DNA replication and genome copy number is greatly increased early during seedling development, resulting in up to 10,000 copies of chloroplast DNA per plant cell in young leaves. However, chloroplast genome copy number decreases as leaf cells mature, as a result of a decrease in DNA replication coupled with continued chloroplast and cell division, although the mechanism for this control is not known. We have localized minimal tobacco chloroplast DNA sequences required for *in vitro* replication activity from both origins, and observed sequence-specific interactions between these sequence elements and proteins from the partially purified tobacco replication fraction. In this continuing project we will use DNase I footprinting to examine the exact DNA sequences which interact with the protein(s). We plan to purify and characterize the protein(s), and examine their specific role in the regulation of initiation of chloroplast DNA replication. The long-term goal is to clone the gene(s) which encode these protein(s), with the objective of determining the mechanism by which chloroplast DNA replication and genome copy number is regulated, and how this relates to the development of photosynthesis in plants. In addition, an understanding of DNA sequence and protein elements which are involved in chloroplast DNA replication may facilitate the construction of vectors for the introduction and expression of foreign genes in chloroplasts.

9701405 Genetic Control of Surface Lipid Biosynthesis in Sorghum
Jenks, M.A. **Grant 97-35301-4964**

Arkansas State University, State University **Strengthening Award**
College of Agriculture **$100,000**
State University, AR 72467 **2 Years**

Sorghum growers, like those of similar crops, suffer significant losses to a variety of pathogenic fungi. Increasing fungicide use by US farmers is increasing the cost of crop production and leading to the accumulation of toxic residues in our soil and groundwater. Fungi are also developing resistance to currently used fungicides. Therefore, alternative methods are needed to protect crops from fungal pathogens. The diverse surface lipid coatings covering all aerial plant tissues likely play a key role in plant resistance to fungi. Genetic engineering should therefore be explored as a means of modifying crop surface lipids to improve crop pathogen-resistance. However, little is known about gene involvement in plant surface lipid biosynthesis, or the role these lipids play in plant pathogen-resistance.

This proposal plans to employ sorghum as a model crop system to elucidate gene involvement in the biosynthesis of surface lipids. Single and double mutants in sorghum will be analyzed using gas chromatography mass-spectrometry to describe substrate flow through, and gene product function in, surface lipid biosynthesis. Sorghum's predominance of free acids on the surface makes it an ideal model for dissecting wax precursor (acyl-CoA) elongation and associated reactions. In addition, these mutants will be employed in field and greenhouse studies to examine surface lipid involvement in sorghum susceptibility to three important crop fungi, *Exserohilum*, *Colletotrichum*, and *Gloeocercospora*. In the future, findings from this research will be used with genetic engineering to modify sorghum surface lipids and improve sorghum's pathogen resistance.

9701412 Mechanism of *a3*, a Negative Regulator of Maize Anthocyanin Genes
Chandler, V.L. **Grant 97-35301-4354**

University of Arizona
Department of Plant Sciences
Tucson, AZ 85721

$237,000
3 Years

A better understanding of the regulation of plant genes is crucial for genetic engineering strategies in important crop species such as maize. This research is to characterize the function and mechanism of action of the *a3* gene, first identified as a recessive intensifier of maize anthocyanin pigment. Our recent results indicate that the *a3* gene encodes a negative regulator acting at transcriptional or post-transcriptional levels. Our data support a simple model in which the *a3* gene product negatively regulates mRNA levels of the *b* and *r* regulatory genes. The *b* and *r* genes encode transcription factors, which directly activate the transcription of the genes encoding the anthocyanin biosynthetic enzymes. Experiments will be done to further test and refine this model. We will use transgenic maize lines containing different regions of the *b* gene to identify the *cis*-acting sequences required for *A3* regulation. We will isolate transposable element insertions in *a3* and use these alleles to clone *a3*. The availability of naturally occurring variants, the ability to now use molecular methods with transgenic maize plants, and the extensive genetic tools available, make this an excellent system for studying gene regulation. Few negative regulators have been well characterized in plants. Therefore, further studies of the negative gene regulation exhibited by *a3* may provide clues to understanding the complex genetic and molecular interactions involved in gene control.

9701922 Modes and Mechanism(s) of Cosuppression Mediated by Sense Transgenes
Jorgensen, R.A. **Grant 97-35301-4220**

University of Arizona
Department of Plant Sciences
Tucson, AZ 85721-0036

$120,000
2 Years

When an extra copy of a gene is introduced to a plant by genetic engineering, often the result obtained is the opposite of the intended result: instead of increased gene expression, the introduced gene suppresses its own expression and that of the original copy of the gene. This phenomenon is known as cosuppression. We have found that there are two kinds of cosuppression. One depends on strong expression of the gene and the other requires that the introduced gene be present in two copies arranged as an inverted repeat. Cosuppression of the first type is a highly non-linear response to a small increase in either gene dosage or the amount of gene expression that inversely amplifies such increases into a dramatic reduction in the abundance of gene product. Although this unintended result can be a problem for genetic engineers, it can also be a benefit, if used appropriately, because cosuppression can be used to turn off genes that are unwanted, for instance to eliminate toxic products or to change the oil composition of a seed. Petunia flower color is very useful trait that can be used for model studies of the mechanism of cosuppression, a primary goal of this project. Ultimately, this information will allow us to understand how to control, use, and avoid cosuppression and so to improve the efficiency and predictability of crop plant genetic engineering. The mechanisms of control of gene expression are also of fundamental importance in plant biology, and have no known parallel in animal biology.

9701250 The Positive Effect of Introns on Plant Gene Expression
Rose, A.B. **Grant 97-35301-4392**

University of California, Davis
Section of Molecular and Cellular Biology
Davis, CA 95616-8535

New Investigator Award
$110,000
2 Years

The information contained in most plant genes is interrupted by non-coding sequences known as introns. Introns are deleted after the gene has been transcribed into mRNA but before the mRNA is translated into protein. Introns are not simply a kind of packaging material that must be removed before the information in a gene can be read. In many examples, the amount of protein derived from a gene is greatly increased by the presence of an intron. Currently, the means by which introns enhance expression are not known.

The goal of this project is to determine the mechanistic basis for the positive effect of introns on the expression of plant genes. Experiments to achieve this goal will be performed with a well characterized and versatile test system consisting of a gene that has been modified to facilitate the quantification of gene expression, and one of that gene's natural introns which can be easily isolated and experimentally manipulated. The main question to be answered is whether or not there are specific DNA elements within an intron that are required to increase expression, or alternatively, whether the process of intron removal is necessary and sufficient for this enhancement.

This research will increase our understanding of a very important but poorly understood fundamental aspect of plant gene expression. A deeper understanding of the factors needed for abundant gene expression could have enormous practical benefit because there are many scientific and commercial applications in which a high level of protein synthesis is desirable.

9701919 Inter-organelle Communication: Signals and Transcription
Larkin, R.M. **Grant 97-35301-4656**

The Salk Institute for Biological Studies **Postdoctoral Fellowship**
Plant Biology Laboratory **$75,000**
La Jolla, CA 92037 **2 Years**

The functional state of the chloroplast affects the transcription of nuclear genes that encode chloroplastic proteins, but the molecular nature of chloroplast-to-nucleus communication is not known. The goal of the proposed research is to identify chloroplastic signals that control nuclear gene transcription and to gain insight into the mechanism of repression of nuclear gene transcription in cells that contain chloroplasts that have been inactivated by photooxidative damage (photobleaching). Biochemical experiments have been designed to identify a factor that affects nuclear gene transcription and responds to the functional state of the chloroplast. The biochemical properties of a factor(s) that is identified by this approach will be studied. These biochemical studies will provide information that will be useful for isolating the gene(s) that encodes this factor. The proposed research will also involve the study of previously identified mutations in one of six nuclear genes (referred to as *gun* for genomes uncoupled) that impair the repression of nuclear gene transcription in photobleached cells. The GUN4 protein is thought to interact with two other GUN proteins or to function in a redundant signaling pathway. Moreover, *GUN4* and other *GUN* genes appear to perform non-overlapping functions during the plant life cycle. Thus, *GUN4* is an important component of this inter-organelle signaling pathway. Experiments have been designed to isolate the *GUN4* gene by genetic methods. The combined biochemical and genetic approaches should help to illuminate molecular mechanisms of chloroplast control of nuclear gene transcription.

9701506 Analysis of the Protein-protein Interactions Required for Efficient Translation
Gallie, D.R. **Grant 97-35301-4404**

University of California, Riverside
Department of Biochemistry **$120,000**
Riverside, CA 92521-0129 **2 Years**

A long standing goal of agriculture has been to increase the protein content of crops. In order to achieve this, a basic understanding of the control of protein synthesis in plants is required. We have shown that the cap and poly(A) tail, both of which serve to increase the stability and translational efficiency of mRNAs, are functionally co-dependent, suggesting that these two mRNA elements communicate during translation. The basis for the interaction involves protein-protein interactions between those eukaryotic initiation factors (eIFs) 4F, eIFiso4F, and eIF4B that bind to the 5' cap and the poly(A)-binding protein (PABP) bound to the poly(A) tail. One consequence of this interaction may be to commit the translational machinery to an mRNA for as long as the physical integrity of the mRNA is maintained. We have found that the phosphorylation of eIF4B is developmentally regulated. We will investigate the role of this phosphorylation during translation to determine whether it plays a role in regulating protein synthesis. These studies will be the first to examine the role of the interactions between the termini of mRNAs in plants as a means by which global control of translation is achieved during seed development and germination.

9702004 High Light- and Blue/UV-A-Regulated Responses in Photosynthetic Organisms
Grossman, A.R.; Briggs, W.R. **Grant 97-35301-4575**

Carnegie Institution of Washington
Department of Plant Biology **$140,000**
Stanford, CA 94305-1297 **2 Years**

In the mid-day sun, photosynthetic organisms absorb an excess of light energy and must be able to dissipate that energy as heat. If they lose this capability, absorbed radiant energy would lead to the formation of highly reactive oxygen species that would destroy the cell. A number of different mechanisms may have evolved to reduce the flow of excess excitation energy into the photosynthetic reaction centers. We are studying a pigment-protein complex in the cyanobacterium *Synechococcus sp.* PCC 7942 that increases in high light. The major pigments in this complex are the xanthophylls, which are thought to be involved in the dissipation of excess absorbed light energy in both vascular plants and green algae. We have already isolated the gene for one polypeptide present in this complex; this polypeptide resembles light-harvesting polypeptides present in vascular plants. Continued dissection of the polypeptide components of the complex, an analysis of the function of the complex in high light grown cells, and elucidation of the mechanism by which light controls the biosynthesis of the complex will give us insights into how plants cope with high light and perhaps even reveal the evolution of function and composition of light harvesting complexes.

9701310 Analysis of Plant U1 snRNP 70K Protein Function in Basic and Alternative Splicing
Reddy, A.S.N. **Grant 97-35301-4219**

Colorado State University
Department of Biology
Fort Collins, CO 80523
$130,000
2 Years

Over eighty percent of protein-coding genes in plants contain non-coding intervening sequences (introns). RNA splicing, a process by which non-coding sequences from precursor messenger RNA are removed, is one of the regulatory events involved in controlling gene expression in eukaryotes. Furthermore, some developmental processes are controlled by alternate splicing of the pre-mRNAs resulting in different protein products from the same gene. U1 snRNP 70K protein, a protein that binds to a small nuclear RNA (U1 snRNA), is believed to play a key role in basic and alternative splicing. Little is known about the biochemical and molecular mechanisms that regulate basic and alternative splicing in plants. We have recently isolated and characterized full-length cDNAs and corresponding genomic clone encoding U1-70K protein from *Arabidopsis*, the only known U1-70K in plants. The plant U1-70K protein is coded by a single gene that produces two different transcripts by alternative splicing of a 910 bp-long included/excluded intron. We propose to study the function of U1-70K protein in basic and alternative splicing in plants using a variety of approaches including yeast complementation, protein-protein interaction based screening and manipulation of this gene in specific tissues of transgenic plants. The proposed studies should provide information on the role of the U1 snRNP 70K protein in basic and alternative RNA splicing in plants and important insights into the extent of unity and diversity in RNA splicing mechanisms between plants and animals. The knowledge derived from such studies should yield new insights into regulation of gene expression in plants.

9701913 Intron-enhanced Gene Expression in Maize
Hannah, L.C. **Grant 95-35301-2080**

University of Florida
Department of Horticultural Sciences
Gainesville, FL 32611
$100,000
2 Years

All life forms are dependent on the proper expression of the thousands of genes located within their cells. Our ability to modify genes through molecular biology, alter levels of gene expression and insert these modified genes through tissue culture provides an avenue for plant improvement. While sequences located at start sites for gene expression receive primary attention, this work concentrates on sequences located elsewhere in genes, in regions termed introns. Introns refer to sequences located within the expressed gene but which are removed before the transcribed RNA is converted into protein. Specific to this proposal, focus is placed on the mechanism by which a particular intron, the first intron of the corn gene termed *shrunken 1*, increases gene expression. First the precise sequences within this intron that are needed for enhanced gene expression will be identified. Secondly, we will determine whether these sequences must lie within the first intron for enhanced gene expression or whether they can reside anywhere within the gene. Finally, we will determine whether these sequences operate in a whole plant as they do in tissue culture cells. This information should then provide us with a tool by which the expression of selected genes can be enhanced 100-fold.

9702010 Regulation of the Maize *C1* Gene
McCarty, D.R. **Grant 97-35301-4424**

University of Florida
Department of Horticultural Sciences
Gainesville, FL 32611-0690
$110,000
2 Years

The genetic variation present in natural plant populations is an essential resource for crop improvement through plant breeding. This project addresses how different versions of the maize *C1* regulatory gene have contributed to the wide variation of pigmentation patterns found in maize and its teosinte relatives. We have used the *C1* gene extensively as a model for dissecting the molecular mechanisms that control seed development in maize. We have shown that another regulatory gene named *Vp1* controls expression of *C1* in the seed and that the VP1 factor binds directly to regulatory DNA sequences contained in the *C1* gene. We find that the sequence differences that account for much of the natural allelic variation of *C1* expression are located in or near the VP1 DNA binding site. In order to understand the regulatory mechanisms that control expression of *C1* alleles we propose the following specific objectives: 1. Analyze the regulation of different forms of the *C1* gene. 2. Construct new *C1* variants by intragenic recombination of existing forms that produce unique patterns of pigmentation.

9701354 Floral Regulatory Elements and New Transposons in Sorghum and Other Grasses
Peterson, T.A.; Chopra, S. **Grant 97-35301-4403**

Iowa State University
Department of Zoology and Genetics, and Department of Agronomy
Ames, IA 50011-3223
$110,000
2 Years

In maize, the *P* gene regulates the production of a red flavonoid pigment in floral organs. In this proposal, we will compare the structure and regulatory properties of the maize *P* gene with homologs in other grasses. The species to be studied include a teosinte (*Zea mays pariviglumis*), a wild relative of corn; and candystripe sorghum (*Sorghum bicolor*). The P-homologous genes from each of the above species will be cloned, the gene structure will be determined, and comparisons made among the individual genes. Using our knowledge of the maize *P* gene as a basis, we can test for the presence, absence or modification of certain regulatory elements which we know, or suspect, are important for regulation of the maize *P* gene. The results of this approach should provide important insights into the evolution of gene regulation. Additionally, the focus on *P* gene homologs will allow the information we obtain to interface directly with research in other laboratories on the development of floral morphological characteristics of cereal species, such as teosinte and maize. Finally, a direct outcome of the proposed research will be the first isolation of an active transposable element from sorghum. This particular transposable element, derived from the sorghum *Y-cs* locus (a *P* gene homolog), has promising characteristics for gene tagging and cloning.

9701407 Molecular Analysis of Meiotic Recombination
Schnable, P.S.; Nikolau, B.J. **Grant 97-35301-4343**

Iowa State University
Departments of Agronomy, and Biochemistry and Biophysics
Ames, IA 50011-1010
$100,000
2 Years

Meiotic recombination is one of the central mechanisms by which genetic diversity can be generated. The resulting genetic diversity is fundamental to evolution and to the improvement of crop species. In addition, determinations of the rates at which meiotic recombination occurs between genes are used to create chromosome maps that often play a critical role in the molecular isolation of genes. Considerable evidence, including our work with maize, indicates that meiotic recombination does not occur evenly throughout the genome, and in fact recombination "hot" spots and "cold" spots can be identified on chromosomes. Until very recently technical barriers precluded the investigation of the molecular mechanisms which determine where recombination occurs on plant chromosomes. However, we have recently molecularly cloned a 460-kb segment of chromosome 3 of maize that encompasses the entire chromosomal interval between the *a1* and *sh2* genes. The unique features offered by this technical achievement will enable us to molecularly characterize the features of meiotic recombination that determine where in the maize genome recombination preferentially occurs, and how genetic factors affect this choice.

9702109 Selection of Mutations in Undefined, Complex Biochemical Pathways
Thornburg, R.W. **Grant 97-35301-4405**

Iowa State University
Department of Biochemistry and Biophysics
Ames, IA 50011
$110,000
2 Years

The preparation and characterization of mutants have been an invaluable tool in understanding complex cellular and biochemical pathways. The goal of this proposal is to select for plants that have been mutated in a biochemically undefined pathway. Analysis of these mutant plants and eventually cloning the genes responsible for these mutations should provide a wealth of new knowledge about inducible gene systems and signal transduction in plants. To accomplish this selection, we have used a molecular genetic system based on the pyrimidine biosynthetic pathway. The work will be done using the plant *Arabidopsis thaliana*. We have chosen the Proteinase Inhibitor II gene (*pin2*) from potato (*Solanum tuberosum*) to conduct this work, because it is wound-inducible in plants and has been the primary focus of the PI's research for many years. The *pin2* promoter has been linked to a negative selectable marker, cytosine deaminase. Thus when the *pin2* promoter is induced *in planta* and the plants are incubated on selective medium, the normal plants will die, but the mutant plants will survive. The successful completion of this work will establish a novel method of producing directed mutations in plant systems. We have chosen to work in *Arabidopsis thaliana* with a wound-inducible gene. However, this scheme is a general method that could be used to select for plants that fail to express any desired phenotype. To use such a selection scheme, all that is needed is a well-defined promoter having the desired phenotype that can be linked to the negative selectable marker gene and expressed in plants. This scheme should work with either constitutive promoters or with other inducible promoters.

9701593 Isolation and Characterization of an *Arabidopsis* Gene for Tolerance to TRSV Infection
Domier, L.L.; Hartman, G.L.; Bent, A.F. **Grant 97-35301-4364**

USDA Agricultural Research Service
Crop Protection Research Unit **$120,000**
Urbana, IL 61801 **2 Years**

Most commercial crop plants are susceptible to infection by several different viruses. Virus infection can lead to significant losses in the amount and/or quality of plant products. Plants that can be infected by a virus are called susceptible and those that can not be infected are called resistant. However, not all susceptible plants develop obvious disease symptoms, i.e., some virus-infected plants develop severe disease symptoms, while other closely related plants display few symptoms and suffer little loss in yield. The term tolerant has been used to describe these healthy looking, but infected plants. The obviously diseased plants have been termed sensitive to virus infection. The long-term goal of the proposed research is to identify the genetic and biochemical mechanisms by which tolerant plants are able to survive and thrive in the presence of virus infection while sensitive plants are severely damaged. The proposed experiments will lead to the isolation and characterization of a thale cress (*Arabidopsis*) gene that provides tolerance to a virus that infects a number of economically important crop plants. A combination of molecular and genetic techniques will be used to determine the function and mode of action of the tolerance gene. The successful completion of this work should facilitate future efforts to identify tolerance genes in other plant species and lead to a better understanding of methods to reduce virus-induced crop damage through traditional breeding and genetic engineering approaches.

9701797 Characterization of Plant Polyadenylation Factors
Hunt, A.G. **Grant 95-35301-2041**

University of Kentucky
Department of Agronomy **$130,000**
Lexington, KY 40546-0091 **2 Years**

The proposed study examines a crucial aspect of gene expression in higher plants, namely the addition of poly(A) tracts to messenger RNAs. A clear understanding of mRNA polyadenylation is important, for a complete appreciation of the growth and development of plants, and for efficient, rapid application of genetic engineering technology to crop improvement. Plants are different from other organisms in specific aspects of mRNA polyadenylation, and the lessons learned from other model systems cannot be readily applied to higher plants. In this study, the genes that encode nuclear poly(A) polymerases in plants will be isolated and characterized. In addition, other factors that participate in the process of mRNA polyadenylation will be purified and studied. These studies will help to clarify the differences that exist between plants and other organisms with respect to mRNA polyadenylation. They will also lend much needed insight into nuclear RNA metabolism in plants. This insight will impact many areas, including susceptibility of plants to RNA pathogens (viruses and viroids) and the use of RNA-based technology (sense, antisense) to develop improved agricultural products.

9701594 Expression of Plant tRNA Genes
Folk, W.R. **Grant 97-35301-4425**

University of Missouri, Columbia
Department of Biochemistry **$110,000**
Columbia, MO 65211 **2 Years**

Plants provide the majority of food and fiber for mankind, and the efficiency of production and the quality of plant materials must be improved to meet the increased demands of consumers. Plant gene expression occurs via three enzymatic machineries. RNA polymerase III transcribes small RNAs utilized in decoding genetic information. Despite the signal importance of tRNAs in plant metabolism, little is known about the control of tRNA gene expression in plants. We propose to identify and purify the enzymatic machinery responsible for tRNA gene expression in plants, and to define how they interact with each other, with the template DNA and with RNA polymerase III. Information from these studies can be used for many applications such as: 1. to provide the means by which to regulate expression of plant proteins. 2. to generate tRNAs capable of miscoding so as to improve protein quality. 3. to improve the utility of tRNA gene to serve as promoters for the expression of ribozymes and antisense RNAs.

9701774 Core and Holo Plant RNA Polymerase II, Subunit Composition and Function
Guilfoyle, T.J. **Grant 97-35301-4363**

University of Missouri, Columbia
Department of Biochemistry
Columbia, MO 65211-0001
$120,000
2 Years

Transcription of nuclear genes in all eukaryotic organisms is carried out by three classes of DNA-dependent RNA polymerases. RNA polymerase II transcribes genes that encode messenger RNAs which are translated into proteins. Plant RNA polymerase II, like RNA polymerase II in animals and lower eukaryotes, contains four subunits related to the core RNA polymerase of prokaryotic organisms. In addition to these four conserved subunits, plant RNA polymerase II contains eight additional polypeptides that form a core enzyme. Recently, more complex forms of RNA polymerase II that contain twenty or more additional polypeptides have been identified in yeast and mammalian cells, and these are referred to as holo RNA polymerase II enzymes. Holo RNA polymerase II, in contrast to core RNA polymerase II, is able to accurately initiate RNA synthesis on genes that encode messenger RNAs and respond to transcriptional activator and repressor proteins that regulate the level of transcription. Specific objectives are (1) to identify and characterize holo RNA polymerase II in plant cells and (2) to determine the function of RNA polymerase subunits in enzyme assembly and transcriptional processes. Our results will provide information on the basic transcriptional machinery in plants and how this machinery functions in transcription of genes that encode messenger RNAs. This information is important for understanding the mechanisms involved in the regulation of specific and diverse sets of plant genes. Furthermore, understanding the basic transcriptional mechanisms in plants will provide insight into the design of novel genes and novel ways to regulate genes in transgenic crops.

9701361 Mechanisms Regulating pol I Transcription and Nucleolar Dominance in *Brassica*
Pikaard, C.S. **Grant 97-35301-4294**

Washington University
Biology Department
St. Louis, MO 63130
$120,000
2 Years

Ribosomes, the cellular "machines" responsible for protein synthesis, are composed of four RNA molecules and approximately 80 proteins. Three of the four required ribosomal RNAs (rRNAs) are cut from a precursor RNA synthesized by the enzyme RNA polymerase I (pol I). There are thousands of copies of the genes that encode the rRNA precursor arranged one after another at specific locations on plant chromosomes. When rRNA genes are active, ribosomes are assembled around these chromosomal sites, resulting in a nuclear domain, visible under the microscope, called the nucleolus.

When two species are crossed to form a hybrid, often the rRNA genes of one parental species form a nucleolus but nucleoli fail to form around the rRNA genes contributed by the other species. This phenomenon, known as nucleolar dominance, was first described in 1928 and occurs in organisms as diverse as plants, insects, amphibians, and mammals. Two sets of mechanisms are probably needed to establish and enforce nucleolar dominance. We have shown that DNA methylation helps repress the inactive set of rRNA genes and is part of the enforcement mechanism. However, the mechanisms that discriminate between parental rRNA genes and establish nucleolar dominance are unknown. Using a cell-free pol I transcription system we have developed, we will test the hypothesis that dominant rRNA genes compete most effectively for proteins required for rRNA gene expression. We will also test the hypothesis that methylation of rRNA genes can prevent these activator proteins from initiating rRNA synthesis.

9701772 Molecular Genetics of Downy Mildew Resistance: The *Arabidopsis Rpp8* Cluster
Dangl, J.L. **Grant 95-35301-1569**

University of North Carolina, Chapel Hill
Department of Biology
Chapel Hill, NC 27599-3280
$100,000
2 Years

We study the molecular basis of disease resistance in plants. Loss of plant harvest yield due to pathogens and continued application of environmentally unsound pesticides are major agronomic problems. It is hoped that direct isolation and manipulation of single genes conferring pathogen resistance will help alleviate these problems. We have chosen, in our USDA funded research, to analyze the infection of a model plant, *Arabidopsis*, with the parasite which causes downy mildew disease. Together with collaborators in the United Kingdom, we are isolating disease resistance genes directed against several different isolates of this pathogen. These genes tend to "cluster" in the plant chromosome region of interest. This means that they are located close to one another and implies that evolution is working through the expansion of multigene families to meet the challenges of the evolution of new pathogen strains. We use a variety of molecular and genetic methods to isolate these genes. In the current project period, we will continue to analyze the first gene in this chromosomal region which we very recently cloned. It is called *Rpp8*. We will determine how *Rpp8* is organized and what its DNA and inferred protein sequences are. We already know that *Rpp8* is structurally related to other plant disease resistance genes directed against fungal, bacterial and viral pathogens.

Rpp8 is also a member of a small multigene family and we will now isolate and sequence the other members to begin to understand how the evolution of new disease resistance genes occurs.

9701353 Nucleotide Diversity in *Brassica oleracea* Floral Homeotic Genes
Purugganan, M.D. **Grant 97-35301-4688**

North Carolina State University **New Investigator Award**
Department of Genetics **$100,000**
Raleigh, NC 27695-7614 **2 Years**

The domestication of crop plant species is usually associated with changes in plant form and structure. The genetic basis behind these transformations in plant morphology, however, remains poorly understood. It appears likely, though, that many of these structural changes result from alterations in genes that regulate developmental systems. Diversity at these regulatory genes can thus be expected to affect the rate at which selection, both natural and artificial, can operate. We will investigate the molecular population genetics of structural variation in floral homeotic genes that control the development of the altered inflorescence architectures within the cole crop species *Brassica oleracea*. Our approach will be to sample alleles for the *CAULIFLOWER* (*BoCAL*) gene, and sequence these alleles from both wild and cultivated subspecies within *B. oleracea*, including cauliflower and broccoli. The extent and patterning of sequence diversity between alleles will be used to learn more about the origin and history of different subspecies, and to examine the degree to which population genetic forces such as mutation, recombination, selection, genetic drift and gene flow interact at these floral homeotic loci. This study will provide information on how domestication affects the structure of genetic diversity of crop plant populations, and permit us to design more rational and targeted strategies for germplasm conservation. This population genetic approach should also furnish insights into the molecular evolutionary bases of morphological variation that characterizes the diversification of crop plant species.

9701311 The Role of a Family of Nuclear-Encoded Sigma Factors in Plastid Transcription Regulation
Allison, L.A. **Grant 97-35301-4514**

University of Nebraska **New Investigator Award**
Department of Biochemistry **$130,000**
Lincoln, NE 68538-0664 **2 Years**

Coordinated expression of photosynthetic components in response to environmental signals such as light has a profound impact on plant efficiency and crop yield. In chloroplasts, the photosynthetic organelles of plants, some genes which encode proteins of the photosynthetic apparatus are expressed in a light-responsive manner. In part, this enhanced gene expression is due to light-regulated RNA synthesis by the chloroplast RNA polymerase. It is still not clear how the RNA polymerase is directed to transcribe specific genes in the light. One model suggests that RNA polymerase selects particular genes by interacting with specific selectivity proteins known as sigma factors. These sigma factors, encoded in the nuclear DNA, may themselves be differentially expressed in response to light or developmental cues. The aim of this proposal is to investigate the role of a family of sigma factors in regulating chloroplast transcription in the model plant, *Arabidopsis thaliana*. Three sigma factor cDNAs have been isolated from this plant and will be used to address the following questions: i) are the encoded proteins targeted to the chloroplast compartment; ii) do they associate with the chloroplast RNA polymerase; iii) are they differentially expressed? In addition, by manipulating (reducing or increasing) the synthesis of each of the sigma factors in *Arabidopsis* we can ask which subset of chloroplast genes requires each of the sigma factor proteins for expression. In this way we will begin to understand how genes in the nuclear compartment of a plant cell can control environmentally-regulated expression of genes in the chloroplast compartment.

9701366 RbcS mRNA Translation and Stability in *Chlamydomonas*
Baker, E.J. **Grant 97-35301-4816**

University of Nevada, Reno **Strengthening Award**
Department of Biology **$100,000**
Reno, NV 89557-0015 **2 Years**

Chlamydomonas reinhardtii, a unicellular green alga, is an excellent model system for the study of a variety of areas of basic cell biology and biochemistry, particularly photosynthesis. The ability to genetically alter both the chloroplast and nuclear genomes of this organism has allowed powerful analyses of gene function not yet possible in higher plants. In order to fully live up to its potential as a model organism, studies addressing the fundamental molecular biology of gene expression must keep pace with genetic analysis. Control of the process of translation (protein synthesis) is a key area for investigation. The amounts of specific proteins synthesized can be controlled by intrinsic differences in the abilities of different messenger RNAs (mRNAs) to be translated, and by differences in the stabilities (lifetimes) of the mRNAs serving as templates for translation. The molecular bases for these differences are not well understood. The major project seeks to understand the basis for the striking translational inefficiency of an mRNA encoding a protein subunit of the major chloroplast enzyme, ribulose bisphosphate carboxylase, RbcS2.

We propose to determine which nucleotide sequences are critical for its translational inactivity by using recombinant DNA techniques to alter RbcS2 gene sequences to produce modified mRNAs *in vivo*. We have also determined that the stability of RbcS2 mRNAs can be altered by growth in different carbon sources. The second project seeks to understand the molecular basis for the carbon source-regulated stability of this mRNA and its possible relationship to translational efficiency.

9701313 Apocarotenoid Biosynthesis and Function in Plants
Schwartz, S.H. **Grant 97-35301-4427**

University of Nevada, Reno
Department of Biochemistry
Reno, NV 89557-0014

Postdoctoral Fellowship
$ 80,000
2 Years

Apocarotenoids are derived from the oxidative cleavage of carotenoids. These molecules are widely distributed in nature and serve important biological functions in diverse organisms. Vitamin A, for example, is necessary for development and vision in animals. In plants, abscisic acid (ABA) serves a role in seed development and adaptation to a variety of stresses. Many additional apocarotenoids have been identified in plants, but their functions remain speculative. The biosynthesis of apocarotenoids is catalyzed by enzymes that oxidatively cleave carotenoids. There is evidence for this type of enzyme in organisms from all five kingdoms, but little progress has been made in their characterization. Recently, the gene encoding a cleavage enzyme for ABA biosynthesis has been cloned. The identification and characterization of this gene has provided the basis for identifying additional carotenoid cleavage enzymes. The primary goals of this project are to elucidate the function of apocarotenoids in plants and to clone the genes necessary for their synthesis.

9701348 Investigation of the *Sw-5* Gene for TSWV Resistance in Tomato
Tanksley, S.D. **Grant 97-35301-4422**

Cornell University
Plant Breeding and Biometry
Ithaca, NY 14853

$160,000
2 Years

We propose to perform a comprehensive analysis of the *Sw-5* gene conferring resistance to tomato spotted wilt virus (TSWV). In doing so, questions pertaining specifically to TSWV resistance and to disease resistance mechanisms, in general, will be addressed. Among the questions we hope to answer are: 1) What is the structure and function of the *Sw-5* protein product? 2) What is the expression pattern of *SW-5*?) Is *Sw-5* a member of a dispersed or clustered multigene family and do *Sw-5* homologs correspond to any known tomato R genes? 4) Is a single gene at the *Sw-5* locus responsible tor broad spectrum tospovirus resistance? 5) Is *Sw-5* capable of providing resistance in other crop plants (e.g., lettuce)? 6) Can additional resistance alleles be found in wild *Lycopersicon* species and what DNA sequence differences distinguish resistant and susceptible alleles? Examination of these issues should yield information regarding the molecular genetic mechanisms of disease resistance, the conservation of these mechanisms within and between plant families, the evolutionary process by which R genes arise and assume new specificities, and the best means of developing plants with increased disease resistance.

9701367 Genetic Components Required for Paramutation at the Maize *pl* Locus
Hollick, J.B. **Grant 97-35301-4430**

University of Oregon
Institute of Molecular Biology
Eugene, OR 97403-1229

New Investigator Award
$ 140,000
2 Years

The genesis, maintenance, and manipulation of genetic variation are of primary agronomic importance. Yet the very nature of genetic variation is poorly understood. This work addresses an example where variation is exhibited by a single gene. The *pl* (*purple plant*) gene in corn controls the production of purple pigment. One allele (a particular form) of the *pl* gene can exhibit a range of activities conferring weak to intense coloration. Particular levels of activity are heritably changed through interactions with the other *pl* allele; this influence of one allele on another is called paramutation. Thus, using certain combinations of *pl* alleles, plant color can be either enhanced or reduced. This is reminiscent of the processes of inbreeding depression and heterosis (hybrid vigor); it is expected that these studies on an experimentally amenable pigment gene will lend insights into the mechanistic basis of both processes. The long term goal of this work is to elucidate the molecular basis of these allelic interactions that occur during paramutation; what DNA sequences are required and what cellular proteins mediate the process. As a first step, mutations affecting *pl* paramutation have been identified. Some of these mutations reside within the *pl* gene and others reside in different genes; together these mutations address the above goals. The primary aim of this project is to further characterize these mutations and their effects on paramutation. Results of this study will identify molecular models which can subsequently be tested. A thorough understanding of this process should eventually lead to novel approaches for both traditional and marker-assisted breeding strategies.

9701568 1997 Gordon Research Conference on Epigenetic Effects on Gene Expression
Matzke, M.A. **Grant 97-35301-4342**

Gordon Research Center
Gordon Research Conferences **$5,000**
West Kingston, RI 02892-0984 **1 Year**

The 1997 Epigenetics Gordon Research Conference is the second in this series, and judging from the response to the first, should attract an enthusiastic group of scientists. Epigenetics is one of the most exciting frontiers in genetical research. A formal definition of epigenetics would be that it deals with changes in gene expression, particularly gene silencing, that are brought about by potentially reversible changes in DNA methylation or chromatin structure. A simple way to think about epigenetics is that it comprises the "gray" aspects of genetics, i.e., the genes involved do not always conform to the black and white Mendelian laws of inheritance. Examples include genes that are expressed only when they are inherited from either the male or the female parent ("parental imprinting"), genes that are continually silenced for one or more generations (paramutation), and genes that exhibit continuous variation in expression levels (variable expressivity). The universal nature of epigenetic phenomena is apparent from the diverse effects that have been discovered in organisms ranging from single-celled yeasts and filamentous fungi to higher plants and animals. Epigenetics has implications for plant breeding and adaptation as well as human development and disease. Understanding the causes of epigenetic gene silencing is of particular interest for the agricultural biotechnology industry, because unwanted inactivation of new genes introduced into genetically engineered varieties is frequently a problem. The Epigenetics Gordon Conference is unique in bringing together researchers working with various epigenetic phenomena from different organisms, thus permitting interactions among scientists who might not normally meet.

9701771 Interaction and Organization of Zeins in Maize Protein Bodies
Coleman, C.E. **Grant 97-35301-4222**

Brigham Young University **New Investigator Award/Strengthening Award**
Department of Botany **$100,000**
Provo, UT 84602 **2 Years**

Zeins are proteins produced in maize grain that are used by the seed to store nitrogen and amino acids for the germinating seedling. Approximately 50-60% of seed proteins are zeins. These proteins do not contain lysine or tryptophan, which are essential amino acids in the diet of humans, non-ruminant livestock and poultry. The reduced amount of these amino acids makes the grain nutritionally deficient. Zeins are packaged as spherical bodies within the cells of the seed's nutritive tissue. Disruptions in the way zeins are packaged lead to profound changes in the physical properties of the seed, all of which are detrimental to the commercial viability of the grain. It is the purpose of this project to study the interactions between zein proteins that lead to the formation of the spherical protein bodies. The project is also designed to learn whether the zein proteins can be altered, specifically by adding lysine and tryptophan amino acids, without significant interruption of their packing arrangement. These goals will be accomplished by transferring normal and altered forms of the zein genes into tobacco plants. Interactions between the zein proteins in the seeds of these tobacco plants can then be studied without interference from native proteins. The results from these experiments will provide a basis for future work of genetically introducing modified zein proteins into maize seeds in order to manipulate the balance of amino acids.

9701391 The Plant Mitochondrial *nad1* Gene/*mat-r* Gene Complex
Wolstenholme, D.R. **Grant 97-35301-4689**

University of Utah **Strengthening Award**
Department of Biology **$100,000**
Salt Lake City, UT 84112-0840 **2 Years**

The gene (*nad1*) for subunit 1 of the mitochondrial NADH dehydrogenase complex (complex I of the respiratory chain) of maize is interrupted by four introns of the group II kind. One of these introns contains a gene (*mat-r*) for a protein related to yeast group II intron maturases, and this intron and two others are themselves interrupted by large sequences that include other genes. Consequently, different segments of the *nad 1* gene (exons) and the associated half introns are separately transcribed. It is postulated that these transcripts must join either by hydrogen bonding between complimentary sequences, or by end-to-end covalent linkage to generate complete group II introns that can be correctly excised to produce functional transcripts. We propose to define the exact ends of transcripts that contain the two halves of each split intron in order to determine what sequences could be included in the reassociated intron, and whether observed, short repeated sequences might mark the locations at which the intron sequences were divided by recombination. A further series of experiments will be carried out to determine whether it is likely that *trans*-splicing of split introns and *cis*-splicing of the fourth, continuous intron of the maize *nad1* gene follow a similar pathway to that of yeast mitochondrial and plant chloroplast group II introns that are known to involve a lariat-like intermediate.

9701375 Genetic and Molecular Approaches to Modifying the Composition of Seed Oils
Browse, J.A. **Grant 97-35301-4426**

Washington State University
Institute of Biological Chemistry
Pullman, WA 99164-6340
$227,000
3 Years

Many plants accumulate seed reserves as oils composed of triacylglycerols. These vegetable oils constitute important sources of food and industrial products with commercial production worth $25 billion worldwide. To a large extent, the properties of different oils depend on the degrees of unsaturation of the fatty acid components, and this is a function of desaturase enzymes that introduce double bonds into the fatty acid chains. There is considerable interest among plant breeding and biotechnology companies in producing modified oil crops, which would help to diversify farm economies and to advance sustainable agricultural systems. Conventional and mutation breeding have provided some desirable alterations in the composition of several oilseeds. Our work has used mutants of *Arabidopsis* with alterations in seed fatty acid composition to study oilseed metabolism and to clone key desaturases that determine oil composition. Most recently, we have cloned a series of desaturases from plants and the nematode *Caenorhabditis elegans*. This project will employ these genes to bring about useful modifications in oil composition. The work will give us the opportunity to investigate the ability of seed biochemistry to accommodate the changes in fatty acid structure that occur. Recent examples indicate that understanding and altering the enzymatic machinery of oilseeds will be a key to successful engineering of oil composition.

9701356 The Structure and Replication of the Plant Mitochondrial Genome
Bendich, A.J. **Grant 97-35301-4428**

University of Washington
Botany and Genetics Departments
Seattle, WA 98195-5325
$70,000
2 Years

Our knowledge of how the mitochondrial genome in plants is replicated is poor, as is our understanding of the structure of this genome *in vivo*. We do not know whether mitochondrial DNA (mtDNA) replication occurs primarily by an expanding bubble (theta-form) or linear concatemer (as in a rolling circle or a multi-fibered network) mechanism. Despite having circular genomic restriction maps the mtDNA extracted from plants contains very few or no genome-sized, or large circular molecules. Instead, the mtDNA analyzed by pulsed-field gel electrophoresis (PFGE) is composed of linear molecules about 50-150 kilobase pairs in length, much longer linear molecules and complex DNA structures larger than the genome size that remain in the well (origin) of the gel after electrophoresis.

The overall objective is to learn the structure of the plant mitochondrial genome *in vivo* and how this structure is influenced by mtDNA replication. We will investigate mtDNA replication at the higher level of structure where the number of DNA molecules (of enormous molecular weight) approaches the number of segregating genetic units in a mitochondrion. Thus we may elucidate the "ploidy paradox": many copies of the genome but few copies that direct heredity.

We will study the mtDNA from cultured liverwort cells and white mustard (*Brassica hirta*) seedlings in order to: 1) Determine which forms of mtDNA separable by PFGE are generated during mtDNA replication; and 2) Analyze the well-bound mtDNA after PFGE to determine whether it contains rolling circles, single-stranded regions, or highly-branched linear molecules.

The data generated will be useful in choosing and designing mitochondrial genotypes for agricultural improvements, such as for cytoplasmic male sterility.

9701923 Stability of Genetic Redundancy in Allopolyploids
Comai, L. **Grant 97-35301-4429**

University of Washington
Department of Botany
Seattle, WA 98195-5325
$101,886
2 Years

Allopolyploids plants, hybrid species that incorporate two or more diploid sets of parental genomes, are successful both in evolutionary and economic terms. Crops fundamental to human sustenance such as wheat, cotton, oat, the Brassicas, sugarcane, and several tree fruits are allopolyploids. Although the *de novo* synthesis of allopolyploids is possible, new allopolyploids plants usually display genome instability and poor fertility, probably caused by irregular meiosis. These problems are absent in established allopolyploids, which presumably have evolved mechanisms to stabilize their genomes and to achieve a reliable, diploid-like meiosis. My goal is to elucidate the molecular mechanisms important to the success of allopolyploids. This knowledge would facilitate the improvement of many crops. In preliminary work, I have demonstrated gene silencing in the allopolyploid *Arabidopsis suecica* and, from the parents *A. thaliana* (thale cress) and the related *Cardaminopsis arenosa* I have constructed a synthetic allopolyploid. I propose to use established and synthetic strains of *A. suecica* to: i) characterize the extent, cause and role of gene silencing and modulation; ii) elucidate the role of DNA repair proteins in genome stability and chromosome pairing. In the first objective, gene silencing will be surveyed by mRNA fingerprinting. In the second objective, I will test the

hypothesis that DNA repair proteins are responsible for preventing genome instability and irregular meiosis. This hypothesis, supported by a large body of research in bacteria, yeast and animals, will be tested by analyzing genomic rearrangements in the synthetic allopolyploid and by comparing DNA repair in adapted and non-adapted allopolyploids.

9701693 Heterosis among Isolated Strains of the Golden Glow Maize Population
Coors, J.G. **Grant 97-35301-4423**

University of Wisconsin, Madison
Department of Agronomy
Madison, WI 53706-1597
$70,000
2 Years

Hybrid vigor or "heterosis" is the dramatic increase in productivity that occurs when different plants or animals are crossed. In corn, grain yield of hybrid varieties usually exceeds the mean of the parents by more than 50%. Not surprisingly, the phenomenon of hybrid vigor has inspired the creation of a tremendously successful and profitable seed industry. Unfortunately, the genetic mechanisms underlying heterosis are largely unknown, and breeders still have no predictive tools to routinely select the most productive crosses. Firmly establishing the genetic basis for heterosis in corn is an essential goal of geneticists and breeders. This project involves an extensive field evaluation and molecular genetic analysis of three strains of an open-pollinated maize variety, Golden Glow, that have been under selection since the early 1970's. One strain has undergone selection for multiple ears (a component of grain yield not involving heterosis), and the other two were paired in a selection program to increase grain yield when the two strains are crossed (increased heterosis). We will estimate genetic effects directly responsible for the long-term genetic advance in these strains. We have designed the study to separate those genetic factors contributing to heterosis from those that do not. Our results will contribute to the understanding of heterosis and help breeders design more effective breeding strategies.

9701380 Molecular Analysis of Ubiquitin-dependent Proteolysis in Plants
Vierstra, R.D. **Grant 97-35301-4218**

University of Wisconsin, Madison
Department of Horticulture
Madison, WI 53706
$ 240,000
3 Years

Much of plant physiology, growth, and development is controlled by the selective degradation of intracellular proteins. To understand how plants degrade proteins, we are studying a major proteolytic pathway involving the highly conserved, 76-amino acid protein, ubiquitin. In this pathway, chains of ubiquitin are covalent attached to proteins targeted for breakdown. These chains then serve as recognition signals for selective degradation of the target protein by the 26S proteasome, a large proteolytic complex specific for ubiquitinated intermediates. Using *Arabidopsis thaliana* as a model, we will investigate the selectivity of the pathway through a molecular characterization of two important recognition steps. The first step determines which proteins are modified with chains of ubiquitin and involves a key enzyme called ubiquitin protein ligase or E3. Our study will focus on one *A. thaliana* E3 that we have recently isolated in an attempt to determine how an E3 works mechanistically and what target proteins it recognizes. The second step involves the recognition of ubiquitin-protein conjugates by the 26S proteasome. We have recently isolated one such recognition factor call Mcb l for multiubiquitin chain-binding protein. It is a subunit of the proteolytic complex that can recognize ubiquitin assembled in chains. Mutants in Mcb 1 and the E3 will also be isolated and characterized in an effort to identify times in *Arabidopsis* growth and development when these two proteins are essential. Collectively, the data will help reveal how plants selectively degrade proteins and provide strategies for controlling protein breakdown when it interferes with agricultural productivity.

PLANT GROWTH & DEVELOPMENT

Panel Manager - Dr. Gayle K. Lamppa, University of Chicago
Program Director - Dr. Liang-Shiou Lin

This program area supports research aimed at increasing our basic understanding of mechanisms underlying the regulation of plant growth and development in order to achieve optimal productivity of agriculturally important crop plants and forest species. Research areas emphasized by the program include: (1) developmental regulation of gene expression, (2) mechanism of cell division, expansion and differentiation, (3) development and organization of meristems, (4) photomorphogenesis, (5) cell biology, including cytoskeleton, membrane biology, organelle development and cell wall structure, (6) metabolism related to growth and development, (7) hormonal regulation of growth and development, and (8) analysis and control of growth patterns.

9701278 Genetic Analysis of *Arabidopsis* Receptor-like Kinases Using Insertional Alleles
Tax, F.E. **Grant 97-35304-4708**

University of Arizona **New Investigator Award**
Department of Molecular and Cellular Biology **$90, 000**
Tucson, AZ 85721 **2 Years**

In multicellular organisms, cells communicate information to other cells during development. These signals can determine the specific function of cells. While there has been experimental evidence for intercellular signaling in plants for more than 20 years, the specific molecular mechanisms involved have not been well characterized. In animals, receptor protein kinases play key roles during development by responding to extracellular growth factors and regulating intracellular transduction pathways. A large number of plant proteins resembling animal receptor kinases have been found, but since no ligands have yet been conclusively identified, these have been called receptor-like kinases (RLKs). More than twenty different RLKs have been found in various sequencing projects in *Arabidopsis* (mouse-eared cress), but the specific functions of only two or three are known from the analysis of mutants. One way to identify the role of a gene is to identify a mutation in that gene, To determine the function of some of these additional RLKs in *Arabidopsis*, a reverse genetics method has been used to identify insertions in four different RLKs. The goal of the proposed work is to analyze these mutations and characterize the phenotypic consequences of these insertions.

9701255 Maize Inflorescence Development
Hake, S. **Grant 97-35304-4571**

USDA Agricultural Research Service
Plant Gene Expression Center **$100,000**
Albany, CA 94710 **2 Years**

Plant meristems offer an opportunity to study the processes involved in the regulation of determinacy. The maize inflorescence meristem is particularly useful for studying determinacy as it undergoes a series of branching events to produce meristems with increasingly restricted fates. Barren inflorescence mutants are barren of all branches and flowers, as if primordia fail to initiate or become determinate precociously. We propose the following experiments. I) We will carefully describe normal maize inflorescence development using microscopy and *in situ* hybridization. We plan to isolate the maize homolog of *centroradialis* from snapdragons, which is expressed only in inflorescence meristems, and the maize homolog of *leafy* from *Arabidopsis*, which is expressed in inflorescence meristems. II) We will analyze mutants that exhibit precociously determinate phenotypes. We will examine material introgressed into a uniform genetic background with a battery of tools, such as double mutant analysis, microscopy, and *in situ* hybridization. The work in maize offers an additional perspective to the knowledge gained from *Arabidopsis* and *Antirrhinum* floral development since maize has additional meristem stages and is an excellent model organism for understanding floral development in the other agronomically important grasses. The immediate goal is to determine how *kn1* and other floral genes interact in maize floral development with the long range goal of understanding how the switch from an indeterminate to determinate pattern of growth is regulated.

9701297 Fertilization-independent Endosperm Development
Fischer, R. **Grant 97-35304-4941**

University of California, Berkeley
Plant Biology Department **$185,000**
Berkeley, CA 94720-3102 **3 Years**

The long term objective of this proposal is to understand the molecular mechanisms that control plant reproduction and the production of seed and fruit. In the flower, fertilization of the female egg and central cell by male gametes initiates the formation

of seed (embryo, endosperm, seed coat) and fruit development. We use a genetic approach to identify and isolate the key genes that regulate plant reproduction. To this end, we have isolated a novel set of mutations in the model plant system, *Arabidopsis*, named "*fie*" for "fertilization-independent endosperm". In *fie3* mutant plants, in the absence of fertilization, seed-like structures are formed that contain an endosperm surrounded by a seed coat. In addition, *fie3* fruits grow and mature without fertilization. This indicates that the *FIE3* gene regulates a key early step in seed and fruit development. To test this hypothesis, and to begin to understand how fertilization initiates reproduction, we will clone the *FIE3* gene. This will be accomplished by high resolution mapping of the *FIE3* gene relative to previously cloned molecular markers. After the *FIE3* gene is cloned, we will study its function by analyzing its pattern of expression, and by observing the phenotype of *Arabidopsis* plants with altered patterns of *FIE3* expression. These experiments will provide information and tools that can be used to generate better plant varieties by increasing the efficiency of seed and fruit production.

9701534 Regulation of Cell Wall Polymer Substrate Accessibility in Ripening Fruit
Bennett, A.B. **Grant 97-35304-4627**

University of California, Davis
Department of Vegetable Crops
Davis, CA 95616
$120,000
2 Years

Fruit cell wall polymers are disassembled during ripening. This reduces the structural integrity of the fruit and contributes to textural changes, to increases in pathogen susceptibility and ultimately to the complete deterioration of the fruit. We have identified a particular cell wall component which appears to be the earliest to undergo disassembly at the onset of fruit ripening and its disassembly is specifically correlated with the initiation of fruit softening. Experimental results suggest that this specific cell wall component may become accessible to enzyme action at the onset of ripening by the action of a protein referred to as "expansin". An expansin gene was identified that is activated at the onset of ripening and is thus a likely candidate to regulate early events in ripening-associated cell wall disassembly. This research project will test the hypothesis that this expansin gene mediates ripening-regulated disassembly of specific cell wall components by characterizing the protein encoded by this gene and determining its potential cooperative activity with cell wall hydrolases to disassemble cell wall components. The research will be valuable in elucidating a new role for expansin proteins in cooperative cell wall disassembly and will critically test the hypothesis that expansins regulate substrate accessibility and fruit ripening-associated cell wall disassembly. This information will provide the molecular genetic tools to modify this process and to improve fruit quality by controlling fruit softening and deterioration.

9701429 Characterization of a New Class of Genes Involved in Abaxial-adaxial Polarity of the Carpel
Bowman, J.L. **Grant 97-35304-4878**

University of California, Davis
Section of Plant Biology
Davis, CA 95616-8671
$90,000
2 Years

Cereals, legumes, nuts, and fruits of angiosperms provide a large portion of the food that is consumed by both humans and livestock. Each of these products is ultimately derived from carpels, the female reproductive organs of the flower. Carpels are generated in developing flowers through the action of many genes. The aim of this project is to characterize at the molecular level genes that play a role in determining abaxial-adaxial (outside-inside) polarity in the carpel. These genes are likely to be part of the general machinery by which cells acquire their fate in the carpels. Knowledge of the molecular mechanisms that plants utilize to generate pattern and tissues within the carpel may ultimately be used to manipulate the development of these organs. Since all angiosperms are likely to utilize a common set of regulatory genes, by cloning and characterizing these genes in the model species *Arabidopsis*, we can apply the knowledge gained to commercially important crop species. Genetically engineered changes in sizes, numbers, structure and rate of maturity of carpels will eventually be possible. Such manipulations in crop species may lead to improvements in the amount and diversity of our food supply.

9701438 Regulation of Cell Division in Root Growth
Doerner, P.W. **Grant 95-37304-2228**

The Salk Institute for Biological Studies
Plant Biology Laboratory
La Jolla, CA 92037
$150,000
2 Years

Root growth, mediated by meristems, is stimulated by the availability of mineral nutrients. Localized nutrient deposits promote root apical growth as well as branch root formation. Cell division control is a key effector mechanism for the quantitative control of meristem activity. Cell division activity in eukaryotes is controlled by cyclin-dependent kinase subunits in association with cyclins, together comprising the CDK complex. We have previously shown that increased cyclin abundance enhances root growth, presumably by the acceleration of cell division. This suggests that the mechanisms controlling cyclin transcription rates

are involved in root growth regulation, and raises the possibility that cyclin transcription is an effector mechanism for mitogenic signaling. This project focuses on the dissection of the mechanisms controlling cyclin abundance in the root apical meristem and on the pathways that signal nutrient availability involved in cyclin activation.

Molecular approaches will be used to identify the DNA sequences necessary for quantitative control of cyclin transcription. The functional properties of these sequences will subsequently be examined in transgenic cell suspension cultures and plants. Regulatory sequences identified by these methods will then be used as probes to molecularly clone the responsible factors. Physiological data indicate that the availability of mineral nitrogen has strong effects on apical meristem activity, specifically, low levels of mineral nitrogen stimulate root apical growth. The relationship between nitrogen availability and cyclin transcription will be examined. Moreover, we will examine the potential role of plant growth regulators as mitogenic signaling molecules in relation to nutrient availability.

9701276 An Analysis of Floral Regulatory Genes in Maize
Schmidt, R.J.; Yanofsky, M.F. **Grant 97-35304-4659**

University of California, San Diego
Biology Department **$100,000**
La Jolla, CA 92093-0116 **2 Years**

This project addresses the long term goal of understanding the molecular mechanisms underlying flower development in an agriculturally important grass species like corn. Flower development is a complex process involving the apparent interactions of a number of genetically defined loci. We anticipate that a fundamental understanding of the regulation of these developmental processes will lead to the design of novel approaches for engineering beneficial characteristics important to flower development using recombinant DNA technology. In this proposal we are focusing our efforts on three genes that by sequence analysis and pattern of expression are likely to play an important role in specifying the identity of floral organs within the grass spikelet. Mutations in these genes are being sought using reverse genetics, and we will perform a detailed characterization of the effect of these mutations on the development of maize floral organs.

Plant genetic engineering offers the possibility of manipulating plant gene expression. It is essential that we understand the intricate details of the processes of gene expression and regulation in those plant parts that we wish to modify if we are to exploit this new technology to generate new plant types with any degree of predictability. This project will continue our research efforts to unravel the molecular mechanisms of flower development in an agriculturally important cereal species like maize.

9701281 Regulation of Suspensor-specific Gene Expression
Goldberg, R.B. **Grant 97-35304-4740**

University of California, Los Angeles
Department of Cell, Molecular, and Developmental Biology **$95,000**
Los Angeles, CA 90095-1606 **2 Years**

Crop production begins ultimately with seeds that are either produced or purchased by the farmer. For many crops, such as canola and corn, an increase in either the number and/or size of the seed will have a significant impact on yield and crop production. Little information exists on the mechanisms that control seed formation in plants. The seed consists of an embryo, which is responsible for producing the next plant generation, and non-embryonic tissues that protect and nourish the embryo as it develops. An important region of the embryo, designated as the suspensor, provides a novel opportunity to dissect the mechanisms controlling embryo formation during seed development. The suspensor anchors the embryo to the surrounding non-embryonic seed tissues and is thought to serve as a conduit for nutrients from the maternal plant to the developing embryo. The objective of this project is to understand the mechanisms responsible for the regulation of genes that are specifically expressed within the suspensor. To accomplish this objective, genes that are active only within the giant suspensor of the Scarlet Red Runner Bean, *Phaseolus coccineus*, will be sequenced and dissected in order to identify DNA regions that control their activity within the suspensor. These regions will then be used to identify regulatory proteins and genes that are required to switch these genes on during early seed development. The significance of this project is that it should provide new information on the mechanisms that control the differentiation of a major region of the plant seed.

9701293 Elucidation of the Programmed Cell Death Program in Developing Maize Endosperm
Young, T.E. **Grant 97-35304-4657**

University of California, Riverside **Postdoctoral Fellowship**
Department of Biochemistry **$82,000**
Riverside, CA 92521-0129 **2 Years**

During maize kernel development, the endosperm undergoes a cell death program such that at maturity, the endosperm is comprised of non-living starchy endosperm cells surrounded by a single layer of living cells which have differentiated into the aleurone layer. I have carried out preliminary analyses to characterize programmed cell death (pcd) in maize endosperm and found

that cell death initiates within the central endosperm and progresses from the cap toward the base as the endosperm matures. The onset of pcd is preceded by an increase in ethylene synthesis. During cell death, the DNA underwent internucleosomal fragmentation, a hallmark of apoptosis. Ethylene treatment of developing kernels promoted these characteristics of cell death, whereas treatment with an inhibitor of ethylene biosynthesis reduced them, suggesting that ethylene may play a role in triggering the onset of pcd in developing maize endosperm. I will begin the elucidation of steps in the ethylene signal transduction pathway by isolating the ACC synthase gene(s) to use as a tool to identify factors responsible for regulating its expression. I will also isolate the gene(s) encoding the nuclease responsible for the pcd-associated DNA fragmentation to use as a tool to identify intermediate steps in the endosperm pcd process. These studies will provide a starting point to understand the mechanisms responsible for pcd during maize kernel development and may lead to our ability to improve endosperm quality and yield by increasing endosperm life.

9701262 Molecular Genetic Studies of a Near-lethal Abscisic Acid Response Mutant
Finkelstein, R.R. **Grant 97-35304-4875**

University of California, Santa Barbara
Department of Molecular, Cellular and Developmental Biology **$90,000**
Santa Barbara, CA 93106-9610 **2 Years**

The plant hormone abscisic acid (ABA) regulates many aspects of plant growth and development affecting crop productivity, including embryogenesis, water relations and tolerance of a variety of environmental stresses. Our goal is to identify components of ABA signal transduction pathway(s) by isolating and characterizing mutants with altered sensitivity to ABA. The experiments described in this proposal will further characterize the most severe ABA-insensitive (*abi*) mutant we have isolated to date. While most of the known ABA response mutants are highly pleiotropic, none are required for all ABA responses tested or appear to affect essential genes. One of our more recently isolated ABA-insensitive mutants, *abi8*, is a near-lethal, suggesting that it may be disrupting essential functions. Preliminary physiological characterization suggests that ABA may affect root growth by controlling sugar mobilization in this sink tissue. We plan to continue characterizing the physiological effects of the *abi8* mutation and clone the affected gene. The physiological characterization of this mutant will form the basis of future molecular studies of the *ABI8* gene product following cloning of the gene. This will contribute to both our understanding of ABA action and the functional map of the *Arabidopsis* genome. A better understanding of the mechanism of ABA action should enable us to improve stress tolerance, seed quality and viability of synthetic seeds of a variety of crops. Finally, we expect these studies to have broad significance to the field of signal transduction, either as additional examples of current paradigms or by providing evidence for novel signaling mechanisms.

9701286 Regulation of *Arabidopsis* Floral Homeotic Gene Expression
Irish, V.F. **Grant 95-37304-2226**

Yale University
Department of Biology **$95,000**
New Haven, CT 06520-8104 **2 Years**

The *APETALA3* gene is required for specifying petal and stamen identities in the *Arabidopsis* flower. Consonant with this role, the *APETALA3* gene is expressed only in those regions of the floral meristem that will give rise to the petals and stamens, initially just in a region of about eight to ten cells. This proposal aims to identify those factors which are involved in activating *APETALA3* expression in this limited domain. Using genetic and molecular screening approaches, we will identify new genes which are responsible for the appropriate regulation of *APETALA3*. We have generated transgenic plants which harbor a construct containing the petal-specific control element of *APETALA3* driving the *DTA* gene, which confers cell lethality. These plants lack petals, but are otherwise normal and fertile. We will recover second-site mutations which disrupt this lethality; such mutations represent lesions in genes required to activate *APETALA3* expression. In addition, using staged tissue, we will identify genes which are expressed prior to, or coincident with, *APETALA3*. These genes are good candidates for being involved in regulating aspects of *APETALA3* expression. The identification of genes required to activate *APETALA3* in a spatially limited domain, and by extension, required to regionalize the meristem, will be a critical step in understanding how meristematic cell populations become differentiated. In turn, these analyses should be valuable in developing methods to control and manipulate plant reproduction.

9701321 Mechanism of Light and Developmental Control of Gene Expression in *Arabidopsis*
Wei, N. **Grant 95-37304-2291**

Yale University
Department of Molecular, Cellular, and Developmental Biology **$95,000**
New Haven, CT 06520-8104 **2 Years**

The ability to respond to the light environment is essential for plant growth and development. Higher plants are equipped with a sophisticated signaling network to sense the light condition around them and to modify growth for optimal use of available light energy. Many light-modulated plant responses are well characterized, and in most cases the responses have been shown to require activation of specific genes. A number of light responsive elements (LREs) from promoters of light-regulated genes have been identified that are necessary for mediating light responsive gene expression. *Arabidopsis HY5* locus has been genetically defined as a light signaling regulator of plant development, and it encodes a small bZIP type of transcription factor. Our preliminary data showed that HY5 protein can specifically binds to one of the LREs *in vitro*. This may suggest that HY5 regulates plant light responses by directly binding to the promoters of light-regulated genes. I propose to further characterize the interaction of HY5 and the light responsive promoter elements and to elucidate their roles in the light control of gene expression. This information should contribute to our understanding of the regulatory mechanisms of plant light response at the molecular level. The knowledge gained from this research will be vitally important for the ultimate goal of developing genetically superior or desirable varieties of crop and forest species.

9702006 The *Arabidopsis* GF14/14-3-3 Gene Family: Structure and Function
Ferl, R.J.; Sehnke, P. **Grant 97-35304-4942**

University of Florida
Department of Horticultural Sciences **$90,000**
Gainesville, FL 32611-0690 **2 Years**

The purpose of this work is to gain an understanding of the reason why plants have a family of proteins that, until recently, were thought to be specialized proteins found only in animal brains. Recent data indicate that these proteins are involved in general regulatory control of cellular processes and signal transduction. It is possible that the presence of these proteins in plants indicates a role in fundamental cellular processes that are common among plants and animals, and our goal is an understanding of the fundamentally conserved structure and functions of these proteins. It is possible that these proteins have unique and specialized functions in plants, and emerging evidence indicates roles in the regulation of flower and fruit development and the production of secondary compounds. With a thorough understanding of the structure and function of these proteins we should be able to understand several important biochemical pathways in plants, and possibly modify fruit development for increased production or changes in the composition of secondary compounds without the need for increased culture, harvest or post-harvest investments.

9701533 Molecular and Genetic Analysis of Fiber Differentiation
Ye, Z.-H. **Grant 97-35304-4434**

University of Georgia
Department of Botany **$91,300**
Athens, GA 30602-7411 **2 Years**

Fiber cells in vascular plants have strong secondary wall thickening and, in most cases, heavy lignin deposition. Fibers provide structural support and protection for the plant body. Differentiation of fibers is controlled both spatially and temporally during plant growth and development. This is thought to be mediated by interactions of at least three hormonal signals, i.e., auxin, GA and cytokinin. Since the mechanisms of fiber formation evolved at early stages of vascular plant evolution, studies of the mechanisms controlling fiber differentiation will provide an excellent opportunity to address questions related to both evolution and cytodifferentiation. Due to the lack of suitable tools, the molecular details of fiber differentiation have not been well studied. To address this question, we have used the model plant *Arabidopsis* to screen mutants with altered fiber distribution. In wild-type stems, fibers form an arch-shaped pattern in interfascicular regions. The formation of interfascicular fibers is well correlated with the timing of stem maturation and of needed strength for support of shoots. We have isolated an *Arabidopsis* mutant which lacks normal interfascicular fibers. As a result, the mutant stems are brittle and cannot stand erect. Further characterization of this mutant and cloning of the gene will lead to a better understanding of the molecular mechanisms controlling fiber differentiation. Since plant fibers have many economic uses, such as in paper making, cordage and textiles, understanding the underlying mechanisms of fiber differentiation would not only enrich our knowledge of basic scientific aspects of differentiation but also have both economic and agronomic implications.

9701912 Coordinate Regulation of the Acetyl-CoA Carboxylase Genes during Seed Development
Nikolau, B.J.; Wurtele, E.S. **Grant 97-35304-4969**

Iowa State University
Department of Biochemistry and Biophysics, and Department of Botany **$90,000**
Ames, IA 50011-0001 **2 Years**

During the development of plant seeds large quantities of fatty acids are synthesized and stored within the seed. These seed-storage products constitute the vegetable oils that represents one of the major agricultural products of this country. Acetyl-CoA carboxylase is one of the enzymes that are required for the biosynthesis of fatty acids. This enzyme appears to have a critical role in controlling fatty acid biosynthesis and hence seed oil deposition. The acetyl-CoA carboxylase that is involved in fatty acid biosynthesis is a product of four genes. In *Arabidopsis*, these are the *CAC1*, *CAC2* and *CAC3* genes, which are located in the nuclear genome, and the *accD* gene, which is located on the plastidic genome. We have isolated and sequenced all four of these genes. The proposed experiments are designed to elucidate whether and how the expression of these four genes is coordinated during the development of seeds. Achievement of this goal will provide a molecular understanding of the regulation of seed oil biosynthesis *via* the control of acetyl-CoA carboxylase gene expression. Such an understanding is essential for a rational molecular genetic improvement of oil seed crops.

9701418 Blue Light Regulation
Kaufman, L.S. **Grant 95-37304-2290**

University of Illinois, Chicago
Department of Biological Sciences **$95,000**
Chicago, IL 60612-7227 **2 Years**

Plants require light to initiate and maintain their normal developmental process. The light is perceived by photoreceptors sensitive to both the wavelength and intensity of the light as well as its special location on the plant. Upon capture, the light energy is converted to a biochemical signal and results in the activation of one or several signal transduction mechanisms. It is the interacting cascades of signaling mechanisms that eventually results in the initiation and maintenance of the plant's developmental programs. Two major classes of photoreceptor participate in plant development, the phytochromes, absorbing primarily red and far red light and the blue UVA receptors absorbing almost exclusively blue and UVA light. Activation of either class of receptor results in the immediate induction of several nuclear-coded gene families. One such gene family, *Lhcb* (light-harvesting chlorophyll-binding), encodes the protein responsible for binding most of the chlorophyll observed in a plant. Treatment of dark grown pea or *Arabidopsis* seedlings with a single short (less the 10 sec) pulse of low fluence (less than one second of full moonlight) blue light will result in the activation of specific members of the *Lhcb* gene family. This induction occurs in the absence of protein synthesis. The same light treatment results in the activation of a heterotrimeric GTP-biding regulatory protein. G-proteins are well characterized and recognized as signaling compounds in both plants and animals. The goal of this proposal is to define the relationship between activation of the G-protein and the induction of the *Lhcb* gene.

9701425 The Regulation of Ethylene Biosynthesis in *Arabidopsis*
Kieber, J.J. **Grant 95-37304-2294**

University of Illinois, Chicago
Department of Biological Sciences **$90,000**
Chicago, IL 60607 **2 Years**

The gaseous hormone ethylene has profound and agriculturally significant effects on plant growth and development. The enzymatic steps involved in the biosynthesis of this hormone have been elucidated and the corresponding genes isolated. Here we propose to continue our investigation of the mechanisms regulating ethylene biosynthesis in etiolated *Arabidopsis* seedlings. Employing a simple seedling response to ethylene as a facile genetic screen, we identified a number of mutants that are affected in the regulation of ethylene biosynthesis. These mutants either have elevated basal levels of ethylene production (*Eto*s) or fail to increase ethylene biosynthesis in response to the plant hormone cytokinin. Our studies of these mutants have led to the hypothesis that post-translational modifications of ACC synthase, which catalyzes the rate-limiting step of ethylene biosynthesis, play an important role in regulating ethylene production. Furthermore, we have found that cytokinin regulates ethylene biosynthesis through one member of the *ACS* gene family, *ACS5*, in *Arabidopsis*. The goals of the work proposed here are to continue our studies of the *Eto* mutants and to determine how *ACS5* is regulated by cytokinin. We will attempt to clone the *ETO1* gene to learn more about how this gene regulates ethylene biosynthesis. ACC synthase will be characterized from wild-type and *Eto* mutant plants to determine the nature of the modification of these proteins in the mutants. We will examine the properties of the ACS5 protein from seedlings grown in the presence and absence of cytokinin. These studies should provide further insight into the mechanisms regulating ethylene biosynthesis.

9701416 Molecular and Developmental Analysis of Shoot Growth of *A. thaliana*
Pickett, F.B. **Grant 97-35304-4658**

Loyola University of Chicago
Department of Biology
Chicago, IL 60626

New Investigator Award/Strengthening Award
$90,000
2 Years

The genetic regulation of the development of plant embryos residing within seeds is largely unknown. By identifying mutants that disrupt normal embryo formation in predictable ways, we have identified genes that promote normal embryogenesis when present in their wild-type, non-mutant form. The identification of the proteins encoded by these genes will provide insight into the molecular mechanisms that guide cells in the embryo from a naive state, to a state in which the cells can play appropriate roles in the formation of leaves, stems and flowers. We will identify novel embryonic genes by exposing plants to mutagenic chemical and physical agents, and by screening in their offspring for the physical manifestations of these gene mutations. In addition to our mutant screens, we have begun to produce a fate map of the embryo of *Arabidopsis*, a cruciferous plant related to broccoli, cauliflower and canola. The fate map will provide the time and location during embryonic development when cells encounter regulatory cues that cause them to participate in the formation of the seedling leaves (cotyledons) and the meristem. The meristem acts as an enduring embryo, producing new organs throughout plant development. All of the visible structures of plants, including stems, leaves and flowers, develop when naive cells in the meristem receive regulatory cues. Thus our fate mapping and genetic analysis comprise a combined approach to first characterize developmental programs in the embryo and then analyze the molecular mechanisms that organize and direct these developmental programs.

9701531 Profilin Function during Maize Pollen Germination and Tube Growth
Staiger, C.J. **Grant 97-35304-4876**

Purdue University
Department of Biological Sciences
West Lafayette, IN 47907-1392

$90, 000
2 Years

Pollen is the major vehicle for successful transmission of genetic information from a parent plant to the next generation. The pollen grain functions by projecting a cytoplasmic extension, the pollen tube, for delivery of the gametes to the embryo sac where they accomplish double fertilization. Polarization of the cytoplasm, selection of a germination site and tube extension all depend upon the actin cytoskoleton. Profilin is a ubiquitous monomeric actin binding protein that has complex effects on actin polymerization, and is an abundant component of plant pollen. The biochemical characterization of pollen profilin has been initiated and the indications are that profilin has the potential to be an intermediate between signal transduction cascades and actin reorganization.

This proposal aims to dissect the function of profilin during maize pollen development using cytological, biochemical and molecular approaches. Our hypothesis is that profilin is a key modulator of actin organization in pollen, and that loss of profilin function will result in failure to reorganize the actin cytoskeleton with consequent disruption of pollen germination and tube extension. Moreover, we believe that the multiple profilin isoforms in maize pollen are functionally distinct and that they regulate actin organization at discrete locations in the cell. By these studies we will provide critical insight into the mechanisms used to control actin organization and dynamics and into how the actin cytoskeleton is rearranged in response to external signals. The findings will dramatically improve knowledge of pollen biology and provide tools for future manipulation of plant sexual reproduction.

9701692 Role of Multiple Phospholipase Ds in Plant Reproduction and Senescence
Wang, X. **Grant 97-35304-4877**

Kansas State University
Department of Biochemistry
Manhattan, KS 66506-3702

$210,000
3 Years

Phospholipase D (PLD) is an enzyme cleaving phospholipids which are the backbones of biological membranes. This enzyme has been proposed to be involved in many cellular processes including cell proliferation, reproduction, and response to the environment. Three PLDs have been cloned from *Arabidopsis* and they are different gene products. Genetic manipulation studies have indicated that these PLDs may have different functions in plant growth and development. To elucidate how the PLDs function differently, this project will characterize the catalytic and regulatory properties of the cloned PLDs. Specifically, the substrate specificities of and calcium effects on each PLD will be determined. To understand what their functions are, plants deficient in one, two, or three PLDs will be produced and analyzed for the effects of these alterations on seed production and post-harvest senescence. The seed production will be investigated because of the observations that suppression of certain PLDs has resulted in partial sterile plants and that a yeast reproductive mutant is caused by the disruption of one PLD gene function. This study may lead to identification of an important regulatory step in plant reproduction. PLD in senescence will be examined since

PLD degrades membrane lipids and may constitute a key step in plant deterioration. Knowledge gained from this study may lead to innovative strategies for crop improvement.

9701396 Ethylene-response and Senescence-related *Arabidopsis* Mutants
Larsen, P.B. **Grant 97-35304-4921**

University of Maryland, College Park
Department of Plant Biology
College Park, MD 20742-5815
Postdoctoral Fellowship
$90,000
2 Years

Ethylene is a simple gaseous hormone that plays numerous roles in plant growth and development, including fruit ripening and senescence. Significant progress has been made in understanding how a plant cell perceives ethylene and how this signal is transmitted to ultimately result in the initiation of complex physiological responses, yet large gaps in our knowledge exist. *Arabidopsis thaliana* (wall cress) has served as an excellent model organism for identification of genes that encode proteins involved in ethylene perception. These include several potential ethylene receptors and other intermediate components of the signal transduction pathway. These genes have been identified through the isolation of *Arabidopsis* mutants that have defects in ethylene perception as assessed by an ethylene-mediated phenomenon known as the seedling triple response. The specific goal of this proposal is to isolate additional components of the ethylene signal transduction pathway through the identification of *Arabidopsis* mutants that have alterations in other ethylene-mediated processes. Mutants that either have an increased sensitivity to ethylene (as assessed by root growth) or that have altered patterns of leaf senescence (a physiological process shown to be mediated by ethylene) will be identified. Ultimately, these mutants should allow for the isolation and characterization of presently unidentified genes that encode proteins that comprise the ethylene signaling pathway. Isolation of these genes should lead to a more complete understanding of hormone perception in plants along with additional opportunities for controlling ethylene responses, an important issue in agriculture and more specifically, post-harvest technology.

9701591 Diversity of Vacuolar H+-Pumping ATPases in Plants
Sze, H. **Grant 97-35304-4523**

University of Maryland, College Park
Department of Plant Biology
College Park, MD 20742-5815
$90,000
2 Years

Acidification of intracellular compartments by a vacuolar-type H+-ATPase (V-ATPase) is central to many cellular processes in plants. This H+ pump provides a major driving force for the uptake and release of ions and metabolites across the vacuolar membrane. As vacuoles represent the largest organelle in mature plant cells, the function of the H+-ATPase contributes directly to cell elongation, regulation of gas exchange and thus the growth, development and adaptability of plants in a changing environment. In spite of its significance, we do not understand what regulates the expression, synthesis and activity of multiple genes encoding this proton pump. One approach to understand these questions is to determine whether expression of multiple genes encoding the major subunits is tissue-specific and developmentally regulated. We are testing whether four distinct genes encoding the 16 kD subunit in *Arabidopsis thaliana* are differentially expressed using analyses of promoter-glucuronidase (GUS) reporter gene fusions in transgenic plants. To understand the biogenesis of this complex pump, we are studying the role of molecular chaperones in the folding and assembly of the H+-ATPase complex in oat roots using subunit-specific antibodies and immunoprecipitation. These studies will help us understand how endomembrane acidification by the V-ATPase in various cell types is integrated into the growth, development and adaptability of plants.

9701409 Signals, Chaperones and Receptors Required for Peroxisomal Protein Transport
Olsen, L.J. **Grant 97-35304-4922**

University of Michigan
Department of Biology
Ann Arbor, MI 48109-1048
$95,000
2 Years

Peroxisomes are amazing organelles. They are found in nearly all eukaryotes, are bounded by a single membrane, and contain enzymes to metabolize hydrogen peroxide (hence the term 'peroxisome'). Distinct classes of peroxisomes are present at different developmental stages of the plant life cycle. These organelles perform a variety of cellular functions that appear to be dictated by the physiological requirements of a specific cell/tissue type. We are currently studying the mechanisms of protein import into glyoxysomes, a unique class of peroxisome prevalent in germinating seedlings. The long-term goal of my research is to understand the molecular mechanisms by which proteins are targeted to and transported into plant peroxisomes. In this proposal, I have focused on questions regarding specific components of the translocation process. First, we will determine whether protein conformation affects the import competence of peroxisomal proteins. Second, we will examine the role of molecular chaperones, especially hsp70 and hsp90, in peroxisomal protein import. Third, we will clone and characterize an *Arabidopsis* homolog of a receptor for peroxisomal proteins possessing a carboxyl-terminal targeting signal. This project will provide significant basic

information about peroxisomal protein transport in eukaryotes. Knowledge obtained from these studies may be directly applicable to the design of strategies to improve crop plants using genetic engineering techniques to target proteins to peroxisomes. Moreover, several neurological disorders in humans result from defects in specific events during peroxisome assembly. Thus an understanding of the mechanisms by which proteins are imported into peroxisomes will ultimately impact broader biological issues.

9701247 Molecular Genetics of Root Hair Initiation in *Arabidopsis*
Schiefelbein, J.W.; Masucci, J.D. **Grant 97-35304-4580**

University of Michigan
Department of Biology
Ann Arbor, Ml 48109-1048
$110,000
2 Years

The regulation of the size and shape of plant cells is critical for proper growth and development. The formation of root hairs in *Arabidopsis* provides a useful model for molecular genetic studies of plant cell morphogenesis because the root hair cells are easily examined and manipulated throughout their formation, and mutants affecting root hair formation can be easily identified and characterized. In the present project, root hair initiation is used to define the molecules controlling a specific change in cell morphogenesis. A mutant has been isolated in *Arabidopsis*, called *rhd6*, that alters the frequency and location of root hair initiation. In preliminary studies, the *RHD6* product has been incorporated into a simple model for the control of root hair formation. The experiments in the present project are designed to test and extend this model, and they include (1) the molecular cloning of the *RHD6* gene, (2) the analysis of *RHD6* in root hair formation, (3) the molecular relationship of *RHD6* to other root hair genes, (4) the cellular analysis of root hair initiation, and (5) the identification of new hair initiation mutants. Because hair formation is the first morphological event to occur exclusively in cells that have adopted a root hair cell fate, the study of root hair initiation offers the opportunity to define the molecular mechanisms that link the adoption of a particular cell fate to the implementation of a specific morphogenesis program. In addition, this work may lead to novel strategies to manipulate root hair production for the acquisition of water and nutrients by plant roots.

9701523 Conference on Plant Cell Biology
Green, P.J.; Somerville, C.R.; Quatrano, R.S. **Grant 97-35304-4725**

Michigan State University
MSU-DOE Plant Research Laboratory
East Lansing, MI 48824-1312
$7,000
1 Year

This meeting will emphasize how studies on cell organization, regulation of cellular processes, and cell-cell interactions mediate plant development. Although most speakers will emphasize cell biological approaches to these problems, molecular, genetic and biochemical perspectives will also be represented.

In contrast to more specialized plant meetings held recently, this conference will integrate studies on the whole plant cell. Presenting a cohesive picture of the plant cell and how plant development is controlled at the cellular level is not the goal of large general plant meetings (e.g., Plant Biology 97 or the International Plant Molecular Biology meetings) or meetings that concentrate on only one species. Even a large conference like the annual Cell Biology Meeting is not expected to cover adequately the unique features of the plant cells such as plasmodesmata, the cell wall, and totipotency.

In addition to providing an optimal forum for the exchange of scientific information among principal investigators, this meeting is designed to maximize the participation of postdoctoral and graduate trainees. We plan to accomplish the latter by defraying conference costs of the graduate students and postdoctoral students submitting the best abstracts, including short talks by graduate students and postdoctoral students in the program, and providing reading lists in the areas covered by the conference for those trainees desiring additional information. Accordingly, this conference should update trainees as well as established investigators on the increasing number of exciting questions in plant cell biology and encourage novel approaches and interactions to address them.

9701642 Relationship between Genetic and Hormonal Control of Sex Expression in *Cucumis* Species
Grumet, R.; Staub, J.E. **Grant 97-35304-4787**

Michigan State University
Horticulture Department
East Lansing, MI 48824
$90,666
2 Years

The ability to form sexually functional flowers is central to plant reproduction and crop yield. Our goal is to understand the molecular basis of sex expression in plants that produce separate male and female flowers. *Cucumis* species such as cucumbers and melons display a unique array of true breeding sex phenotypes controlled by a small number of genes, and thus have served as models for sex expression in plants. In cucumber the dominant F allele which conditions all female flower production has been utilized widely in the production of cultivars with concentrated flowering and fruit set. The differential sex expression also is

controlled by hormonal factors. Application of various plant hormones, especially ethylene, can dramatically alter sex expression. Recent data have demonstrated that an additional copy of a gene encoding a key enzyme in ethylene biosynthesis (ACC synthase) completely co-segregates with the F locus for female sex expression. In the proposed study we will test the hypothesis that sex expression genes can encode enzymes involved in localized regulation of hormone levels. We propose to: 1. clone full length genomic copies of the F-locus and non F-locus associated ACC synthase genes, compare their coding and regulatory regions, and test for tissue specific expression; 2. Test the effect of introduction of the ACC synthase and other genes known to influence ethylene production and/or reception on sex expression in transgenic melon and cucumber plants; and 3. Test the effect of introducing a known sex expression gene from maize (*TASSLESEED2*) into cucumber and melon.

9701384 Effects of Engineered Plant Respiration on Growth/Yield and Low Temperature Response
McIntosh, L. **Grant 97-35304-5109**

Michigan State University
MSU-DOE Plant Research Laboratory **$180,000**
East Lansing, MI 48824 **3 Years**

Plants, but not mammals, have a second "alternative" respiratory pathway in their mitochondria. Its function, in part, is proposed to allow increased synthesis of stress-related compounds. Specific examples of these stresses include: high salt, low phosphate, heat stress, wounding and disease attack. All of these stimuli induce synthesis of the alternative oxidase. Cold stress also inhibits the "normal" respiratory pathway and induces alternative pathway respiration. The alternative pathway may then allow energy production to maintain cell functions and growth under cold conditions. Transgenic tobacco and potato plants and cell lines have been constructed where the alternative oxidase gene is either suppressed or over-expressed. Transgenic cell lines over-expressing the alternative oxidase exposed to lowered temperatures grow at higher rates relative to control cell lines. Cell lines with engineered reduction of alternative oxidase grow much more slowly than controls. Our experiments are aimed at confirming these initial observations with rigorous growth and Carbon Use Efficiency measurements in whole plants. Potato tubers over-expressing alternative oxidase also show increased "sweetening", a detrimental storage trait, during cold storage. New transgenic tuber lines with tuber-specific suppression of the alternative oxidase will be tested to determine if they sweeten less during cold storage. If repressed alternative oxidase activity limits sweetening then a number of commercial lines of potatoes will be engineered to suppress alternative oxidase expression.

9701245 Photoperiodic Control of Gibberellin Metabolism in Long-Day Rosette Plants
Zeevaart, J.A.D. **Grant 97-35304-4692**

Michigan State University
MSU-DOE Plant Research Laboratory **$100,000**
East Lansing, MI 48824-1312 **2 Years**

Long-day rosette plants, such as lettuce and spinach, grow as rosettes (no stems) during the short days of winter, and produce stems and flowers (bolt) during late spring and early summer when the days are long. Previous work has shown that production of gibberellins, a class of plant hormones, is promoted by long days and is essential for bolting. The objective of the proposed research is to investigate how the daylength regulates gibberellin production in connection with bolting in rosette plants. The work will focus on expression of the genes for gibberellin 20-oxidase and 3β-hydroxylase. By over-expression of these genes in spinach and a rosette tobacco, it will be determined whether these enzymes are limiting for gibberellin production. The promoter region of gibberellin 20-oxidase will be fused to the reporter gene for the fluorescent protein from jellyfish to determine under which environmental conditions and in which parts of the plant GA 20-oxidase is produced. This new information on the regulation of gibberellin synthesis will make it feasible to genetically manipulate gibberellin production in rosette plants, such as lettuce and spinach, so that they can be grown in summer without producing flower stalks. It may also be possible to produce dwarf varieties of those plants in which small stature is a desirable trait.

9701592 Sixteenth Annual Missouri Symposium
Baskin, T.I. **Grant 97-35304-4567**

University of Missouri
Division of Biological Sciences **$5,000**
Columbia, MO 65211 **1 Year**

This proposal asks for partial support for the 16th Annual Missouri Symposium, entitled "Signs and Roadways: Protein Traffic and the Cytoskeleton." The meeting is sponsored by the University of Missouri's Interdisciplinary Plant Group (IPG); the dates will be April 16 through April 19th, 1997; and the meeting will be held on campus, at the University of Missouri, Columbia.

The objective of the meeting is to provide a forum for the presentation of recent and significant research in two fields of plant cell biology: compartmentation of proteins within the endomembrane system, and the cytoskeleton. Each of these fields is fast moving and currently producing results of considerable interest, and each field has specialized meetings typically on an every-

other-year basis. However, the fields of protein targeting and the cytoskeleton share the conceptual framework of understanding how things move within the cell. Bringing these two groups of investigators together in a meeting, where they will learn from and interact with each other, will enrich each field. The meeting will have sessions on the endoplasmic reticulum, Golgi apparatus, vacuoles, organelle movements, microtubules and actin. The format of the meeting provides for ample time for questions during the formal session, and for informal interactions during the two long poster sessions as well as social functions.

9701287 Isolation and Characterization of Asymmetric Division Genes in *Volvox carteri*
Kirk, D.L.; Miller, S.M. **Grant 97-35304-4690**

Washington University
Department of Biology **$200,000**
St. Louis, MO 63130-4899 **3 Years**

Stereotyped asymmetric cell divisions are essential for many aspects of higher plant development, but analysis of the genes controlling such plant asymmetric divisions is difficult because most mutations that interfere with them lead to chaotic development and death. Therefore, we are using the green alga *Volvox* as a model system to analyze how such asymmetric divisions may be programmed genetically and executed at the cellular level. In *Volvox* asymmetric division is required to produce reproductive cells called 'gonidia,' and therefore mutations of '*gonidialess*,' or '*gls*,' genes are usually fatal. However, by starting with a mutant in which somatic cells are able to take over the reproductive function, we can recover transposon-induced *gls* mutants and then use DNA flanking the transposon insertion to clone the affected gene. Sequencing of the *glsA* gene (which we have cloned in this way) reveals that the GlsA protein is very similar to one associated with the mitotic spindle in animal cells, and that it has two protein-binding domains, one of which ('J') is specific for a protein known to be a component of the cell-division apparatus in animals. We propose to use antibodies to test the hypothesis that GlsA protein is physically associated with the *Volvox* division apparatus, and *in vitro* mutagenesis to test the hypothesis that the J domain is essential for such localization and for allowing GlsA to act to shift the cell division plane. We also propose to isolate and characterize other *gls* genes in the same manner and analyze how their products interact with GlsA.

9701317 Gender Mutants and Male-specific DNA Markers in *Silene latifolia*
Grant, S.R. **Grant 97-35304-4553**

University of North Carolina, Chapel Hill
Department of Biology **$95,000**
Chapel Hill, NC 27599-3280 **2 Years**

The majority of flowering plants are hermaphrodites with both male and female reproductive organs in each flower (stamens and carpels respectively). However a small number of plant species produce flowers in which only one type of sex organ develops. Individual plants of dioecious species are either male or female, producing flowers with either stamens or carpels but not both. The sexes differ at key sex determining genes. The overall goal of this research is to investigate the genetic differences between males and females and to understand how dioecious plants differ from hermaphroditic plants. This project involves characterization of mutant plants derived from the dioecious species white campion (*Silene latifolia*) which have lost male characteristics. White campion is one of a small number of plant species with sex chromosomes that differ in size. As in mammals, females have two similar X chromosomes and males have an X and a Y chromosome. The Y chromosome carries genes controlling male traits such as the ability to develop stamens and to suppress development of carpels. We have selected over 50 plants that have lost male traits following X-ray mutation. The goal of this project is to identify mutant genes on the Y chromosome and define their location as a first step towards molecular characterization of the masculinizing genes. Since these genes control plant fertility, they will have useful applications in agriculture. For example, they can be used to engineer reversible sterility mechanisms in order to prevent the uncontrolled spread of transgenic plants in open field planting and to increase the ease of hybrid breeding in species that normally inbreed.

9701270 Regulatory Mechanisms of Gibberellin Biosynthesis in *Arabidopsis*
Sun, T.-P. **Grant 95-37304-2463**

Duke University
Department of Botany **$90,000**
Durham, NC 27708-1000 **2 Years**

Gibberellins (GAs) are a large family of diterpenoid compounds, some of which are plant growth regulators, controlling such diverse processes as germination, stem elongation, leaf expansion, and flower and fruit development. Our goal is to understand the mechanisms regulating GA biosynthesis in response to light. We isolated the *GA1* gene of *Arabidopsis thaliana* that controls the first committed step in GA biosynthesis. We have also demonstrated that the expression of *GA1* gene is tissue-specific during development. This proposal describes experiments designed to investigate the molecular mechanisms involved in the light

regulation of *GA1* gene expression. Understanding the basic plant biology of GA biosynthesis will contribute to a knowledge base that can be used to improve agricultural crops in the future.

9701956 *emb10*: a Gene with Diverse Roles in Maize Embryogenesis
Clark, J.K. **Grant 97-35304-4691**

University of North Dakota **Strengthening Award**
Department of Biology **$90,000**
Grand Forks, ND 58202-9019 **2 Years**

A mature maize embryo consists of an embryonic plant surrounded by a large seed leaf, the scutellum. Mutant *emb10* embryos are frequently mispositioned within the kernel, their scutella fail to expand, and the embryonic mesocotyl, a specialized region of the embryonic shoot that is important during seedling germination, is stunted. These and other morphological abnormalities suggest that the *emb10* gene may be involved in determining cell shape and the direction of cell division. The goal of this project is to understand the nature of the *emb10* gene and its role in maize embryo development. The course of abnormal embryogenesis in mutant embryos will be studied in sectioned materials in order to infer (by contrast) the role of the normal gene. The *emb10* gene will be cloned and its sequence will be compared with sequence databases in order to determine whether *emb10* corresponds to other genes of known function. Crosses will be made with chromosome translocation stocks and genetic markers which will enhance the effectiveness of the developmental and molecular analysis. This project will add to our knowledge of fundamental cellular mechanisms of plant development. It will provide insight into the genetic regulation of the process of embryogenesis. And since embryonic mesocotyl differentiation is a taxonomically important trait in the grasses while seedling mesocotyl extension is an agronomically important trait in maize, rice and wheat, this project will set the stage for future comparative studies of *emb10* in other cereal species.

9701256 The Role of the *Hcf106* Gene Product in Thylakoid Membrane Biogenesis
Martienssen, R.A. **Grant 97-35304-4566**

Cold Spring Harbor Laboratory **$200,000**
Cold Spring Harbor, NY 11724 **3 Years**

Protein translocation across membranes is an essential process for all living cells. In plants, biogenesis of chloroplast membranes involves many proteins enclosed in the nucleus, and routing of these products to membrane-bound compartments in the chloroplast has received considerable attention over the last few years. Mutants in chloroplast biogenesis include defects in protein translocation, and *hcf106* mutants of maize are blocked in a protein translocation pathway previously thought to be unique to higher plant chloroplasts. However, the emerging molecular and genetic data suggest that other pathways for protein translocation have been highly conserved between bacteria and chloroplasts, consistent with the prokaryotic origin pf these organelles.

Molecular cloning of the *hcf106* gene and recent advances in bacterial whole-genome sequencing have revealed that cyanobacteria and other bacteria have prokaryotic *hcf106* homologs. Biochemical and genetic data indicate that the HCF106 thylakoid membrane protein defines a ΔpH-dependent protein translocation pathway that appears to be conserved in bacterial cells. We will dissect this novel protein translocation pathway in chloroplasts and in bacteria via mutants and reconstituted uptake systems. We will use the HCF106 gene, mutant strains of maize and *E. coli*, antibodies, constructs and sequence information that we have acquired during the previous funding period. Our work with Hcf106 will complement studies in other laboratories concerning the other proteins translocation pathways in chloroplasts to advance our understanding of protein translocation mechanisms in general.

9701299 Glycosylation Codes of Extensin and Gum Arabic Glycoprotein: An Approach to HRGP Function
Kieliszewski, M.J. **Grant 95-37304-2375**

Ohio University, Athens
Department of Chemistry **$95,000**
Athens, OH 45701-2979 **2 Years**

Plant cell walls contain unique glycoproteins hypothetically involved in growth processes like self-assembly of the cell wall, control of extension growth, and disease responses. We propose to test these hypotheses by chemical characterization of cell wall proteins from tomato and *Acacia senegal*. Our strategy involves glycosylation site identification of the glycoproteins and molecular modeling to determine their three dimensional shape and the codes which dictate their glycosylation specifics. This approach will also allow us to predict the structure of these glycoproteins from cloned sequences and help us understand how they assist in wall assembly, disease resistance, and plant growth regulation.

9701369 Occurrence and Synthesis of the Auxin Conjugates of *Arabidopsis thaliana*
Barratt, N.M. **Grant 97-35304-5107**

Case Western Reserve University **Postdoctoral Fellowship**
Department of Biology **$81,000**
Cleveland, OH 44106-7080 **2 Years**

The plant hormone auxin plays a major role in control of plant growth and development, regulating such processes as elongation and directional growth, development of fruit and vascular tissue, and senescence. Agronomic applications of auxins include use in fruit production and as herbicides. The major endogenous auxin in plants is indole-3-acetic acid (IAA). Precise modulation of IAA levels is key to normal plant growth. In most plants, the bulk of IAA exists not as free, active, hormone, but chemically linked or conjugated to other molecules such as amino acids or sugars. IAA conjugates may serve as forms of the hormone for storage or transport, protection from degradation, or inactivation. The abundance and universality of conjugates suggests the compounds are important in regulation of endogenous hormone levels. The diversity of endogenous and synthetic auxin conjugates suggests individual conjugates may play roles specific to a developmental stage or stimulus. Despite this, little is known of the identities or actions of auxin conjugates in any species. This project will first identify the full complement of endogenous conjugates in *Arabidopsis thaliana* (a wild mustard). Second, conjugate formation will be characterized in response to environmental stimuli such as light intensity and drought stress, and in response to other plant hormones with which auxin interacts. The model dicot, A*rabidopsis*, lends itself well to molecular and genetic studies. An understanding of the identities and occurrence of endogenous conjugates in this species will facilitate study of genes and enzymes involved in auxin metabolism.

9701275 Plant Protein Farnesylation: Role in Cell Cycle Control
Yang, Z. **Grant 97-35304-4565**

Ohio State University
Plant Biotechnology Center **$95,000**
Columbus, OH 43210 **2 Years**

The growth of plants and the formation of a plant body are dependent upon the generation of new cells (a cyclic process called the cell cycle) in a controlled fashion. The molecular basis for the control of the cell cycle is poorly understood in plants. The goal of this proposed work is to investigate the role of protein farnesylation and farnesylated proteins in this control. Farnesylation is a post-translational modification by attaching a lipid membrane anchor to the carboxy terminus of proteins. Farnesylated proteins are anchored to the plamsa membrane and become activated for signal transduction. We have preliminary evidence that farnesylation is involved in the control of the plant cell cycle and have identified a Ras-related small GTP-binding protein (RLP1) that is a potential substrate for farnesylation. In the proposed study, we will use inducible expression of a farnsylation-specific inhibitor to define the role of farnesylation in cell cycle control in synchronized tobacco cells. To assess the role for farnesylated RLP1 in the cell cycle control, we will determine whether the farnesylation of RLP1 is correlated with cell cycle progression in tobacco cells. This work may provide useful information of genetically engineering crop plants with improved productivity.

9701261 Regulation of Microsporogenesis in *Arabidopsis thaliana*
Makaroff, C.A. **Grant 95-37304-2246**

Miami University, Oxford
Department of Chemistry **$95,000**
Oxford, OH 45056 **2 Years**

The characteristic of male sterility has been utilized by plant breeders for many years. Lines exhibiting cytoplasmic male sterility (CMS) have been used in hybrid seed production because they allow the mass production of seed without the tedious and costly process of hand emasculation of female seed parents. However, there are also many problems associated with the use of CMS lines, including a lack of CMS in many species and undesirable traits. For these reasons nuclear male sterility (NMS) is now being explored as a possible alternative for use in plant breeding. However, before NMS can be utilized in agriculture, a more complete picture of pollen development is required, including investigations into the molecular, biochemical, and developmental causes of NMS in plants.

This study involves the cellular and molecular characterization of T-DNA tagged lines of *Arabidopsis thaliana* that exhibit defects in meiosis. Cytological studies to determine the specific nature of the defect in two mutants with defects in synapsis will be conducted. The genes responsible for the mutations will be isolated and the role of the proteins encoded by these genes investigated. Results from this study should provide new insights into early steps of pollen development, that could be utilized in the development of new male-sterility systems.

9701530 Mechanism of Sperm Transport during Fertilization in Angiosperms
Russell, S.D. **Grant 95-37304-2361**

University of Oklahoma
Department of Botany and Microbiology
Norman, OK 73019-0245
$100,000
2 Years

Sexual reproduction and fertilization are critical processes in the continued productivity of plant crops, involving precise cellular interactions to produce fertile seed. The current project is to study how the non-motile sperm cells of flowering plants are transported to their female reproductive target cells during double fertilization and how they fuse. Apparently the same molecules that are involved with movement in muscles, namely actin and myosin, are involved in transporting gametes of flowering plants once they are released from the pollen tube. The subject of the current research is the developmental origin of the distributional patterns of these critical molecules in and on the male and female gametes, and the exact mechanism by which gametes are positioned for fertilization. This investigation will involve using live, fixed and frozen material prepared for immunocytochemistry to determine how specific molecules interact, how sperm cells and female reproductive cells present these molecules, and the conditions under which gamete migration and sexual combination occur. This will contribute to understanding how the genetic material of flowering plants recombines during normally-occurring sexual reproduction, help geneticists to incorporate desirable characteristics through the sexual process, and elucidate how the fertilization process can fail. Alterations to the fertilization process may allow wider hybrids or gamete recombinants to be introduced for improving growth, productivity, hardiness, timing of flowering, fertility and resistance to disease, or to target particular sperm cells for fusion with the egg cell.

9701394 Interplay of Synthases and Thioesterases in Medium Chain Fatty Acid Synthesis
Slabaugh, M.B.; Jaworski, J.G. **Grant 97-35304-4521**

Oregon State University
Department of Crop and Soil Science
Corvallis, OR 97331-3002
$100,000
2 Years

Medium-chain fatty acids 8 to 14 carbons in length predominate in seed oils of some plants, notably the New World genus *Cuphea*. Efforts to transfer the medium-chain phenotype to established crop plants have focused on introducing thioesterase (TE) genes cloned from medium-chain-producing, undomesticated species, with the intention to prematurely intercept growing fatty acid chains. The oil phenotypes of transgenic plants, however, have not fully matched those of the plants from which the TEs were cloned, indicating that contributory activities are probably missing. The beta-ketoacyl-acyl carrier protein synthases (KASs) are prime candidates for this role as they are the condensing enzymes that extend fatty acid chains and thus provide substrate pools that can be either extended further or terminated by TEs. We have recently cloned and characterized a class of KASs from *Cuphea wrightii* that are active in extending short- and medium-chain substrates and have chain-length determining activity when combined with medium-chain TEs in transgenic *Arabidopsis thaliana*. We are proposing to study how members of these two gene families interact as determinants of the medium-chain phenotype. The specific objectives of this study are to (1) Alter the ratio of medium-chain KAS to medium-chain TE in transgenic plants in order to discover whether this will drive an increase in a specific fatty acid, and whether the spectrum of medium-chain fatty acids produced is a direct reflection of the substrate pools available to the TE; (2) Characterize a second *Cuphea wrightii* KAS that is likely to have a different substrate range, by transformation of the model plant *Arabidopsis* already carrying a *Cuphea* TE; and (3) Characterize a mutant of *Cuphea viscosissima*, *clm1*, hypothesized to be altered in a KAS with a different substrate range, in order to evaluate its contribution to chain elongation in a medium-chain fatty acid producing system. These experiments will illuminate the interactions between elongation and termination functions, and may have far-reaching implications for synthesis of unusual fatty acids in crop plants.

9701319 Functional Characterization of Two *Arabidopsis* P-Glycoprotein Homologs, AtMDR1 and AtMDR2
Rea, P.A. **Grant 97-35304-4551**

University of Pennsylvania
Plant Science Institute, Department of Biology
Philadelphia, PA 19104-6018
$95,000
2 Years

This project is concerned with determining the functional capabilities of two new membrane proteins whose genes were isolated from the model vascular plant *Arabidopsis thaliana*. Designated AtMDR1 and AtMDR2, these proteins bear a strong resemblance to each other and to the human and rodent *MDR* (multidrug resistance) gene products. The simplest explanation for the existence of *MDR1*-like genes in vascular plants is that they signify transport functions analogous to those mediated by the *MDR* gene products of nonplant cells, namely ATP-energized alkaloid, peptide and/or general lipophile transport. Our main objectives in the next funding period will be to determine the membrane localization of AtMDR1 and AtMDR2 in the intact plant, to elucidate the range of compounds each is able to transport after their expression in yeast and to isolate transposon-tagged *Arabidopsis* mutants for the genes concerned for future physiological investigations. The significance of AtMDR1 and AtMDR2 will depend on their biochemical capabilities but possibilities could include the manipulation of alkaloid sequestration or

extrusion, for example, during allelopathy or the elaboration of antifeedants, peptide processing for intercellular and intracellular signaling during development or pathogen responses, or the maintenance of lipid asymmetry associated with membrane flow, for instance, during the elimination of lipid oxidation products or extrusion of lipophilic toxins. AtMDR1 and AtMDR2's membership of the ATP-binding cassette (ABC) superfamily of transporters most, if not all, of which utilize ATP as a direct energy source for solute transport, points to a diversity of hitherto unexplored, non-proton-coupled, transport functions in plants.

9701358 Biochemical and Biophysical Characterization of *Arabidopsis* MADS Domain Proteins
Krizek, B.A. **Grant 97-35304-4690**

University of South Carolina, Columbia **New Investigator Award/Strengthening Award**
Department of Biological Sciences **$90,000**
Columbia, SC 29208-001 **2 Years**

Molecular genetic studies in the model plant *Arabidopsis thaliana* have identified a family of genes that play important roles in flower development. Two members of this MADS box family, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), are required for the development of petals and stamen in a flower. AP3 and PI form a heterodimer that binds to DNA and is thought to act as a transcription factor, regulating the expression of a set of genes required for organ development. Although AP3 and PI form homodimers, *in vitro* data indicates that the homodimers do not bind DNA. To understand the basis for this DNA-binding specificity, biochemical and biophysical characterization of the proteins is necessary. We will develop an expression system for AP3 and PI so that these methods will be possible in the future. Molecular modeling of the AP3/PI heterodimer, based upon the known structure of another MADS domain protein, will be performed in order to identify amino acids that may be responsible for the ability of the heterodimer but not the homodimers to bind DNA. The role of these amino acids will be investigated by making mutations at these positions and assaying the resulting sequence variants in *in vitro* DNA-binding studies and in vivo for their ability to rescue the respective (*ap3* or *pi*) mutant. Characterization of the interactions between MADS domain proteins that result in DNA binding dimers is a first step towards understanding the regulatory roles of MADS domain proteins in plant development.

9702005 Phytochrome B, Rhythmic Ethylene Biosynthesis and the Biological Clock in Sorghum
Morgan, P.W. **Grant 97-35304-4820**

Texas A&M University
Department of Soil and Crop Sciences **$95,000**
College Station, TX 77843-2474 **2 Years**

We have found that the plant hormone ethylene is produced in sorghum plants in daily, rhythmic peaks. The size of these peaks is 10 times higher in a cultivar (58M) containing a mutation which eliminates the presence of a light quality and duration measuring pigment than in related cultivars without the mutation. Because cultivar 58M flowers quite early and looks like it is growing in shade, we suspect that its over production of ethylene may be responsible for its unusual appearance and behavior. In the proposed research we will: (1) determine the role of production of the hormone (ethylene) in the unusual appearance and behavior of 58M, (2) determine how plants control the production of the hormone (the absence of the pigment in plants which make 10 times more ethylene suggests the pigment regulates production), (3) determine how shade (light rich in long wave lengths) is linked to tall, spindly, weak rooted growth. Both date of flowering in season-dependent crops and the tendency to be tall and spindly in crowed plantings influence yield and the ability to resist stresses like drought. Our mutation-containing cultivar will likely explain how these processes are regulated. Knowing the detailed story should uncover ways to improve crop plants using biotechnology. The target crops for such improvements will be grain crops which are planted in dense stands and tend to grow tall and spindly because of mutual shading.

9701932 Genetic Analysis of New Mutants Affecting Light Signaling and Development
Pepper, A.E. **Grant 97-35304-4693**

Texas A & M University **New Investigator Award**
Department of Biology **$ 95,000**
College Station, TX 77843-3258 **2 Years**

Understanding how light influences plant growth and development is essential to future crop improvement strategies. In order to study the molecular pathways by which light directs plant growth, a combined molecular-biological and genetic approach has been initiated to study light regulation of seedling development (de-etiolation) in the small crucifer *Arabidopsis thaliana*. Previous screens for genetic mutants have led to the identification of genes for a red/far-red photoreceptor called phytochrome (*PHYA*, *PHYB*), a blue/ultraviolet photoreceptor called cryptochrome (*CRY1*), and regulatory molecules located in the nucleus such as *de-etiolated* (*DET1*) and *constitutive photomorphogenesis* (*COP1*). Little information about the possible signaling steps in-between, such as the initial steps in phytochrome and cryptochrome signaling, has thus far been obtained through genetics. Genetic screens for second site (extragenic) mutations that suppress the phenotype of a known mutation have proven to be a

valuable strategy for the elucidation of complex genetic regulatory pathways in bacteria, yeast, and the fruit fly. To further define the pathway for light regulation of seedling development we have isolated extragenic mutations that suppress the phenotype of *det1*. These have been designated *ted1* through *ted5*. In order to more fully understand the role of *TED1* and *DET1* in the context of the molecular pathways that mediate light regulation of seedling development, we propose to identify, and obtain DNA and protein sequence from the *TED1* gene, and to characterize the biochemical and genetic interactions of *TED1* with *DET1* and other known genes involved in light regulation, including *COP1*, *PHYA*, *PHYB* and *CRY1*.

9701930 ABA and Developmental Regulation of the Carrot Lea Gene *Dc3*
Thomas, T.L. **Grant 97-35304-4552**

Texas A&M University
Department of Biology
College Station, TX 77843-3258

$95,000
2 Years

Seeds accumulate and store proteins and oils; these storage components serve as a nitrogen and energy source for the germinating seedling. In addition, seed oils and storage proteins are of immense agronomic importance. Despite the economic value of the seed storage process, we know relatively little about the details of the molecular and cellular events that result in the high level accumulation of these components in seeds but not in vegetative tissues. In this project, we will focus on the DNA sequences, called *cis* regulatory sequences, that control the exclusive expression in seeds of a carrot gene called *Dc3*. We will try and understand how these DNA sequences respond to developmental and hormonal (abscisic acid or ABA) signals. A second aspect of the project is to identify the nuclear proteins, or *trans*-acting factors, that interact with the *cis* regulatory sequences and to determine the protein/DNA interactions required for seed-specific and ABA-regulated gene expression. These experiments will provide detailed information on the *cis*- and *trans*-acting factors that control the *Dc3* gene. One result of this project will be a better understanding of the molecular events leading to seed- and tissue-specific gene expression. In addition, results from this project may afford the opportunity to manipulate the storage process to enhance the quantity and quality of seed protein and lipids.

9701420 Localization of Prolamine mRNAs to the Protein Body Endoplasmic Reticulum
Okita, T.W.

Washington State University
Institute of Biological Chemistry
Pullman, WA 99164-6340

$95,000
2 Years

Seeds provide a major source of dietary proteins for humans and livestock. The storage proteins which dictate the nutritional quality of seeds are deficient in several amino acids that are essential in the diet of monogastric animals. In on-going efforts to improve the quantity and quality (i.e. a better balance of amino acids) of the storage proteins so that cereal seeds provide a better food source, my laboratory has been studying the expression of the rice storage protein genes. Results from previous studies from my laboratory have unequivocally demonstrated that the mRNAs, encoding the two distinct classes of storage proteins observed in rice seeds, are localized to morphologically distinct endoplasmic reticulum membranes where protein synthesis occurs. These observations suggest that these mRNAs are actively transported and sorted to the two distinct endoplasmic reticulum membranes present in rice endosperm tissue. In this grant application, studies are proposed to determine whether prolamine mRNAs, which encode one of the two storage protein classes, are localized on the protein body endoplasmic reticulum membranes by a cellular process independent of protein synthesis or whether prolamine mRNA localization is dependent on synthesis of the encoded polypeptide. This will be accomplished by analyzing the expression of genetically engineered prolamine genes in transgenic rice plants. Studies are also proposed to identify the relevant RNA or peptide sequences that may be involved in the targeting of prolamine mRNAs to these membranes.

9701257 Eighth International Conference on *Arabidopsis* Research
Amasino, R.M. **Grant 97-35304-4554**

University of Wisconsin, Madison
Department of Biochemistry
Madison, WI 53706-1569

$7,000
1 Year

This award is for support for the Eighth International Conference on *Arabidopsis* Research to be held at the University of Wisconsin, Madison WI, June 25-29, 1997. The aim is to provide an opportunity for researchers to exchange ideas on all aspects of *Arabidopsis* research, including genome-related efforts. The conference will include sessions on plant cell growth, signal transduction, hormone signaling, cell signaling, meristem function, adaptation to stress, metabolic regulation, light-regulated development, and cellular structure. Thirty-nine speakers have been invited and another 24-26 will be chosen from the submitted abstracts for short talks. This meeting has over 600 participants and greater than 500 of those will present a poster or talk. The conference will maximize participation of young scientists by inviting relatively young PIs, postdoctoral fellows, and graduate students to give many of the talks.

9701266 Genetic and Biochemical Characterization of the *Arabidopsis* Calmodulin Domain Protein Kinase
Sussman, M.R. **Grant 95-37304-2364**

University of Wisconsin, Madison
Department of Horticulture
Madison, WI 53706

$90,000
2 Years

Because they are incapable of motion, plants must be able to adapt to a wide variety of environmental conditions. One of the mechanisms they have evolved to adapt to this sessile state is a very sensitive system for detecting changes in light quality and quantity, wind, temperature and the presence of pests and pathogens. The molecular mechanism for this sensing mechanism is a change in the intracellular concentration of calcium, and the enzymes understudy in this proposal are the 'receptors' which detect these changes in cytoplasmic calcium. The role of these proteins is being studied using a new procedure of isolating mutant plants which lack the receptor proteins. An analysis of how these mutant plants are altered in their ability to sense changes in the environment should provide important clues and tests for the hypothesized *in situ* role of these proteins. These particular type of calcium sensing receptor proteins are only found in higher plants and protists and seem to be lacking in fungi and animals. This study should thus also provide knowledge which could be used to target new pharmaceutical agents which alter the growth and plants and protists, perhaps without affecting animals and fungi.

9701274 Epigenetic Mechanisms and Changes in Gene Expression Associated with Flowering
van Nocker, S. **Grant 97-35304-5108**

University of Wisconsin, Madison
Department of Biochemistry
Madison, WI 53706

Postdoctoral Fellowship
$90,000
2 Years

The transition from a vegetative to reproductive state (flowering) represents a major developmental event in flowering plants, and is an essential component of agricultural production. Many plants exhibit accelerated floral initiation in response to an extended cold treatment. This response, termed vernalization, helps to coordinate flowering and subsequent seed production with favorable environmental conditions. I propose to identify the molecular mechanism(s) associated specifically with vernalization, using the reference plant *Arabidopsis thaliana* as a model system. Because the process of vernalization is likely associated wich changes in gene expression, this goal will be approached by identifying genes that are differentially expressed in vernalized vs. non-vernalized plants through differential gene cloning techniques.

Characteristics of vernalization, including the requirement for mitosis during the cold treatment and the mitotic but not meiotic transmission of the vernalized state, strongly implicate the involvement of epigenetic phenomena. Recent studies in *Arabidopsis* demonstrate the involvement of genomic DNA methylation in a myriad of developmental events, including the transition to flowering. As a complementary approach to identifying vernalization responsive genes, I will attempt to identify specific changes in genomic DNA methylation between vernalized and non-vernalized plants, which may be associated with changes in gene expression. This will be accomplished by applying an established technique used for the identification of genotype-specific polymorphic marker sequences to the identification and isolation of methylation-dependent, polymorphic sequences.

An understanding of the mechanisms responsible for vernalization-associated floral initiation, in addition to obvious agricultural benefits, will contribute greatly to our understanding of plant development in general.

PHOTOSYNTHESIS AND RESPIRATION

Panel Manager - Dr. Gerald E. Edwards, Washington State University
Program Director - Dr. H. Jane Smith

Towards expanding our knowledge of fundamental aspects of energy capture and utilization by plants, research funded by this program extends from studies on solar energy absorption to analyses of crop productivity. Research supported includes studies on primary events of light absorption, energy transduction and utilization; structure, synthesis, turnover and interactions of components of the photosynthetic and respiratory systems; mechanisms of carbon dioxide absorption and fixation; metabolism and biochemistry of energy-rich compounds; and translocation and partitioning of photosynthates in the whole plant.

9701526 Molecular Mechanisms of Salt Tolerance in Cyanobacteria
Vermaas, W.F.J. **Grant 97-35306-4881**

Arizona State University
Department of Botany, Center for the Study of Early Events in Photosynthesis **$128,000**
Tempe, AZ 85287 **2 Years**

The long-term goal of the project is to determine factors that affect salt tolerance in photosynthetic organisms. Understanding these factors will help to develop crop plant varieties that are able to thrive at increased salinity levels that may occur in irrigated fields. In algae and cyanobacteria evidence has accumulated that enhanced cyclic electron transport around Photosystem I, which leads to light-generated ATP production, is related to increased salt tolerance. In higher plants environmental stresses also have been correlated tentatively with alterations in cyclic electron flow around Photosystem I. In this project cyclic electron flow around Photosystem I and its relevance to salt tolerance will be probed using the transformable, facultatively photoheterotrophic cyanobacterium *Synechocystis* sp. PCC 6803 for which the genomic sequence has been determined. A high salt sensitivity in a *Synechocystis* sp. PCC 6803 strain lacking Photosystem I already has been demonstrated. The involvement of type-1 and type-2 NAD(P)H dehydrogenase (NDH-1 and NDH-2) and of ferredoxin-NADP oxidoreductase (FNR) in cyclic electron flow around photosystem I and their effects on salt tolerance will be determined in various strains, including in a mutant that lacks Photosystem II as well as respiratory oxidases; in such a strain cyclic electron flow around Photosystem I is one of the very few electron transfer pathways that can still function. The results obtained here can be used to guide efforts to increase salt tolerance in higher plants.

9701651 Alteration of Redox Properties in Bacterial Reaction Centers
Williams, J.C.; Allen, J.P. **Grant 97-35306-4525**

Arizona State University
Department of Chemistry and Biochemistry **$130,000**
Tempe, AZ 85287-1604 **2 Years**

Electron transfer reactions play a crucial role in photosynthesis and respiration. The objective of this research is to define the role of proteins in modifying the properties of molecules that undergo these reactions. The work is focused on the reaction center of purple bacteria, which is similar to Photosystem II of cyanobacteria, algae, and green plants, and thus can be used as a model system for the study of reactions common to nearly all photosynthetic organisms. One of our goals is to systematically modify the energy level of the primary electron donor of the reaction center, a bacteriochlorophyll dimer that is one of the critical participants in photosynthetic electron transfer. Interactions such as hydrogen bonding to amino acid residues will be probed by perturbing the protein environment and determining the effects on the electronic properties of the dimer. Another aim is to study the properties of photochemically-generated tyrosine radicals. Harmful processes involving tyrosine radicals are thought to be responsible for the phenomenon of photoinhibition in Photosystem II, resulting in a high turnover of damaged polypeptides. This process does not normally occur in bacterial reaction centers, so we propose to design a reaction center that is capable of generating an amino acid radical by a light-initiated electron transfer reaction, and to use this system to study the properties and consequences of radical formation.

9702004 High Light- and B1ue/UV-A-Regulated Responses in Photosynthetic Organisms
Grossman, A.R.; Briggs, W.R. **Grant 97-35301-4575**

Carnegie Institution of Washington, Stanford
Department of Plant Biology **$30,000**
Stanford, CA 94305-1297 **2 Years**

In the mid-day sun, photosynthetic organisms absorb an excess of light energy and must be able to dissipate that energy as heat. If they lose this capability, absorbed radiant energy would lead to the formation of highly reactive oxygen species that would destroy the cell. A number of different mechanisms may have evolved to reduce the flow of excess excitation energy into the

photosynthetic reaction centers. We are studying a pigment-protein complex in the cyanobacterium *Synechococcos* sp PCC 7942 that increases in high light. The major pigments in this complex are the xanthophylls, which are thought to be involved in the dissipation of excess absorbed light energy in both vascular plants and green algae. We have already isolated the gene for one polypeptide present in this complex; this polypeptide resembles light-harvesting polypeptides present in vascular plants. Continued dissection of the polypeptide components of the complex, an analysis of the function of the complex in high light grown cells, and elucidation of the mechanism by which light controls the biosynthesis of the complex will give us insight into how plants cope with high light and perhaps even reveal the evolution of function and composition of light harvesting complexes.

9701644 Photosynthetic Reaction Center Intervalence Transitions
Schenck, C.C.; Levinger, N.E. **Grant 97-35306-4462**

Colorado State University
Department of Biochemistry and Molecular Biology **$110,000**
Fort Collins, CO 80523-1870 **2 Years**

Since all agricultural production depends ultimately on the ability of photosynthetic organisms to convert sunlight into chemical energy with high efficiency, research that elucidates the underlying mechanisms of energy capture by photosynthetic proteins provides the foundation for modifying, protecting and improving these functions in crop plants. The experiments described in this proposal will be conducted with bacterial reaction centers due to the advanced state of structural and functional characterization of this protein. Given the similarities between the bacterial reaction center and photosystem II, the results will likely be generalizable to crop and forest plants. One of the most important unresolved questions in the study of photosynthetic reaction center electron transfer is the question of the electronic coupling between initial and final state wavefunctions. In the nonadiabatic approximation, the electron transfer rate is proportional to the square of the electronic coupling matrix element. Thus, electronic coupling may be an important determinant of the rapid rates and high quantum yields of forward electron transfer in the reaction center protein. In spite of the importance of the electronic coupling, there are no independent measures of its magnitude. Indirect estimates of the electronic coupling have been obtained from rate measurements, but the logical arguments supporting this claim are unconvincing and essentially circular. Experimental evidence suggests that there are weak intervalence transitions in reaction centers; a quantitative analysis of these bands will obtain values for the electronic coupling directly and independently of the rate measurements. We will use infrared pump/visible-near infrared probe ultrafast spectroscopy to achieve this goal, and test the nonadiabatic hypothesis.

9701473 Isolation and Biophysical Characterization of Site Directed Mutants of Photosystem II
Diner, B.A. **Grant 97-35306-4882**

E. I. du Pont de Nemours & Co.
Central Research and Development Department **$170,000**
Wilmington, DE 19880-0173 **3 Years**

The protein of the Photosystem II reaction center contributes in three ways to green plant photosynthetic electron transport: 1) as a source of amino acids that participate in electron transfer (e.g. tyrosine), 2) as a binding site for electron transport components like chlorophylls, quinones and metals, and 3) as a modulator of the ability of these components to accept or give up electrons. We propose to locate the binding sites of the electron transport components of the reaction center and to determine the mechanism of binding. We will do this by examining the functional consequences of mutations constructed at specific sites in the protein, by looking for secondary mutations and by adding small molecules that restore normal activity to functionally inactive mutants, and by incorporating into the reaction center amino acids containing magnetic nuclei that will allow measurement of the distance between these and nearby oxidized or reduced electron transport components. We will use a variety of optical and magnetic spectroscopies in this work. Specific sites examined will include the manganese cluster, responsible for photosynthetic water oxidation, and the immediate environments of the tyrosines, quinones and chlorophylls that participate in electron transport. Knowledge of how the protein participates in electron transport will provide us with lessons as to how the natural solar cell, that is, the Photosystem II reaction center, functions with an efficiency of energy storage that far exceeds that of artificial devices and will tell us whether it is possible to improve further upon the efficiency of this natural device.

9701964 Allosteric Variants of Maize Endosperm ADP-glucose Pyrophosphorylase Studied in *E. coli.*
Hannah, L.C.; Greene, T.W. **Grant 97-35306-4461**

University of Florida
Department of Horticultural Sciences **$120,000**
Gainesville, FL 32611 **2 Years**

Corn (maize) is a major agricultural product in the United States as well as in the world. Funds awarded here are used to expand on previous studies which identified a genetic variant that increases corn seed weight by 15% without a reduction in seed number. Hence, this variant should give rise to a 15% increase in corn yield. The identified variant alters the biological properties of a key enzyme involved in starch biosynthesis, adenosine diphosphate glucose pyrophosphorylase such that more

starch is synthesized. The increased seed weight arises because starch comprises some 80% of total seed weight. Specifically, studies will focus on identification of further favorable variants by identifying the function performed by the various parts of the enzyme. Studies will also examine interactions between this variant and another one which alleviates some of the adverse effects of corn by products on the environment.

9701962 Molecular Recognition During Electron Transfer on the Oxidizing Side of Photosystem I
Chitnis, P.R. **Grant 97-35306-4555**

Iowa State University
Department of Biochemistry and Biophysics **$114,000**
Ames, IA 50011 **2 Years**

Oxygenic photosynthesis in plants, algae and cyanobacteria is the major source of biological energy and oxygen on the earth. Photosystem I is one of two photosynthetic complexes that convert light energy into chemical form. It accepts electrons from plastocyanin in thylakoid lumen. We plan to study interactions between Photosystem I and plastocyanin. We are using a naturally transformable cyanobacterium as a model organism because of its amenability to molecular genetic studies. We will alter specific regions in Photosystem I proteins and characterize effects of mutations on interaction with electron donor proteins. These studies will enable us to understand the elements of recognition between these proteins and the importance of specificity in the photosynthetic processes. Electron transfer to plant Photosystem I follows a complex mechanism. We will identify the structural basis for complex formation by generating chimeric Photosystem I complexes that will contain cyanobacterial and plant proteins. The proposed multidisciplinary analyses will reveal structural features that are required for electron transfer to Photosystem I. The outcome of this project will increase our knowledge of electron transfer in photosynthesis and will provide information that can potentially be used to genetically manipulate photosynthesis in crop plants.

9701654 Alteration of the Photosynthetic Mechanism in anN_2-fixing Cyanobacterium.
Sherman, L.A. **Grant 97-35306-4880**

Purdue University
Department of Biological Sciences **$110,000**
West Lafayette, IN 47907-1392 **2 Years**

We have isolated a unicellular N_2-fixing cyanobacterium, *Cyanothece* sp., that is capable of metabolic rhythms. When grown under N_2-fixing conditions, the culture fixes N_2 or evolves O_2 with peaks every 24 h and the two processes are 12 h out of phase. We are interested in determining the mechanism by which photosynthesis and N_2 fixation can coexist in the same cell. N_2 fixation is very sensitive to O_2 and the existence of N_2 fixation in such photosynthetic cells provides an opportunity to understand the relationship of these two processes. Our results indicate that Photosystem II (PSII) is the target of the down-regulation of photosynthesis during N_2 fixation. We will be analyzing short-term, as well as long-term, cellular processes that may affect the structure and function of PSII. Our results indicate that the first step in control may be due to the redox state of the plastoquinone pool, which affects the reducing side of PSII. We then hypothesize that a series of short-term alterations in the membrane take place. These involve the overall organization of PSII and PSI, as well as they way in which energy is transferred to the different photosystems. We will look for controlling mechanisms, such as protein phosphorylation, which may mediate these alterations. We will study these processes using a combination of biochemical, biophysical and molecular biological techniques. These studies will provide us with important information as to the way in which the redox state of the quinone pool affects metabolic processes such as photosynthesis and N_2 fixation. These studies can have three important benefits. First, this is an excellent model system for the analysis of resource partitioning. Secondly, an understanding of how N_2 fixation and photosynthesis coexist in the same cell may generate information that will allow us to genetically engineer N_2-fixation genes into plant cells. In this way, we might be able to greatly reduce the level of nitrogen fertilizer that is used for many crops. Finally, these metabolic processes demonstrate characteristics of circadian rhythms. Thus, gene regulation in this organism might provide important information for an understanding of such circadian rhythms in both plants and humans.

9701451 Molecular Mechanisms of Maize C4 Gene Regulation
Sheen, J.-Y. **Grant 97-35306-4591**

Massachusetts General Hospital
Department of Molecular Biology **$180,000**
Boston, MA 02114 **$3 Years**

Monocot plants such as maize, wheat, and rice are the major crop plants in the world. Understanding photosynthetic gene control in these important monocot plants may offer opportunities for crop improvement and applications in biotechnology. We have chosen to study the regulatory mechanisms of the maize C_4 genes in depth because they encode key enzymes inefficient CO_2 fixation, and their structure, expression patterns, and regulatory elements have been well characterized. The goals of this project are to elucidate the molecular mechanisms andintracellular signal transduction pathways controlling the differential expression

of the maize C_4PPDK and C_4PEPC genes. Specifically, we will 1) investigate the molecular mechanisms underlying the stress repression of the C_4PPDK promoter, 2) elucidate the molecular function of a DNA-binding protein encoded by *Dof1* in the regulation of the C_4PEPC promoter, and 3) reveal the molecular basis of the blue light-inducible expression of the C_4PPDK promoter using cloned cryptochrome genes and maize signaling mutants. A combination of molecular, biochemical, cellular, and genetic approaches will be taken to investigate the diverse aspects of photosynthetic gene regulation in higher plants. The elucidation of the complex molecular mechanisms underlying C_4 gene regulation will provide basic knowledge and valuable tools for crop manipulation and improvement.

9701611 Carbon Concentration Mechanism in Plants and Algae
Goyal, A. **Grant 97-35306-4943**

University of Minnesota, Duluth **New Investigator Award**
Department of Biology **$100,000**
Duluth, MN 55812-2496 **2 Years**

By some estimates aquatic photosynthesis contributes more than 70% of the global CO_2 utilization (fixation + deposition of carbon as carbonate for long term storage). Ambient level of CO_2 in water is insufficient for rapid growth of green algae and aquatic plants; therefore mechanisms for concentrating dissolved inorganic carbon (DIC) are present to increase the rate of photosynthesis and to suppress photorespiration, an energetically wasteful process in plants and algae. An unsolved problem in photosynthesis research is how dissolved inorganic carbon is concentrated by aquatic plants and green algae. We have identified two proteins (LCI-45 and LCI-47) that are likely to be involved in active inorganic carbon transport into the chloroplasts, the site of CO_2 fixation. The proposed research is aimed at confirming and characterizing the function of these two proteins. The long term goal of our research program is to understand the structure and function of the components of aquatic DIC-concentrating mechanism(s). An additional goal is to evaluate feasibility of transferring genes encoding components of DIC transport system to C-3 plants of economic importance. A genetically engineered active inorganic carbon transport system may reduce photorespiration that may result in more photosynthetic biomass production and a lowering of the atmospheric CO_2 equilibrium.

9701608 A Thylakoid Membrane Protein Kinase Associated With Light Energy Transduction Kohorn, B.D. **Grant 97-35306-4526**

Duke University
Botany Department **$110,000**
Durham, NC 27708 **2 Years**

All organisms on this planet are dependent upon plants and algae to produce energy from the sun through the process of photosynthesis. Photosynthesis is carried out on lipid membranes called thylakoids within cells, inside chloroplasts. The capture and dissipation of light energy in the chloroplast thylakoid membrane is thought to be regulated by a protein kinase, an enzyme that can activate a function by transferring a phosphate group. Current models suggest that the phosphorylation of a light harvesting complex (LHCII) induces its movement between the photosynthetic reaction centers thereby altering the transfer of captured light energy. This phenomenon has been termed state transitions. There has been little to no progress toward the isolation of chloroplast protein kinases even for those that may be involved in state transitions. We have isolated thylakoid protein Tak1 (Thylakoid Associated Kinase) that appears to be a good candidate for an LHCP kinase that is involved in state transitions. This proposal seeks to understand the role of Tak1 in thylakoid regulation. Genetic and biochemical tools of algae will be used to test current theories of state transitions. This will lead to a better understanding of protein kinases in the regulation of energy transduction in the thylakoid membrane, and to a new perspective on protein phosphorylation in organelles.

9702011 Role of Novel Mannitol Dehydrogenase in Sink Metabolism and Salt Tolerance
Pharr, D.M.; Williamson, J.D.; Conkling M.A. **Grant 97-35306-4539**

North Carolina State University
Department of Horticultural Science **$180,000**
Raleigh, NC 27695-7609 **3 Years**

Salt accumulation in irrigated soils adversely affects the productivity of many important crops, and may ultimately prohibit the sustainability of agriculture at present levels, particularly in arid regions. Thus, creation of crops with both high primary productivity and salt tolerance is of tremendous agronomic import. Plants such as celery which produce and translocate mannitol, a six-carbon sugar alcohol, have several advantages over plants that translocate only sugars: 1) increased salt tolerance due to mannitol's function as an osmoprotectant. 2) high photosynthetic rates, apparently due to intrinsic differences in the synthesis of hexitols versus sugars, and 3) complete mannitol oxidation yields more energy per molecule than sugars. This project deals with the role of the enzyme mannitol dehydrogenase (MTD) in plant growth, development, and salt tolerance. This enzyme has been found in sink tissues of celery, parsley and olive, where, by mediating the oxidation of mannitol to mannose, it appears to regulate mannitol pool size at the level of utilization or turnover. By thus regulating mannitol concentration, MTD is important in regulating growth and salt tolerance at the cellular level. We propose to further characterize MTD regulation, and to directly test

the contribution of mannitol catabolism to growth and salt tolerance. In addition to assessing environmental and metabolic signals regulating MTD gene expression, we will test the effect of disrupting MTDfunction in plants and cultured cells either by 1) using specific inhibitors (substrate analogs) of MTD activity or 2) by genetically engineering plants that no longer express the MTD gene. Ultimately, such findings could allow engineering of plants with the additional advantages of mannitol metabolism.

9701961 Rubisco Phylogenetic Correction
Spreitzer, R.J. **Grant 97-35306-4525**

University of Nebraska
Department of Biochemistry **$110,000**
Lincoln, NE 68588-0664 **2 Years**

Ribulosebisphosphate carboxylase/oxygenase (Rubisco) is the key photosynthetic enzyme that captures carbon dioxide for the production of starch. Although this reaction serves as the only entry point for atmospheric carbon into the biosphere, oxygen competes with carbon dioxide at the same Rubisco active site, reducing net photosynthesis by as much as 50%. If the enzyme could be genetically engineered to increase carboxylation or decrease oxygenation, an increase in plant productivity would be achieved. However, it is nearly impossible to engineer Rubisco in crop plants because the active site is produced by a gene that resides within the chloroplast. In contrast, chloroplast genetic techniques are well developed for the green alga *Chlamydomonas reinhardtii*, and the structure of the *Chlamydomonas* enzyme is quite similar to the crop plant enzyme. In past genetic studies, regions of the Rubisco protein were identified that influence the carboxylase/oxygenase ratio. The present objective is to genetically engineer these regions so that they will be identical to the structure of crop plant Rubisco. Because crop plant Rubisco is better than the *Chlamydomonas* enzyme, it will be possible to identify those regions that account for improvement in the carboxylase/oxygenase ratio. Once identified, these regions would be the appropriate targets for engineering further improvement in crop plant Rubisco.

9701605 Excitation Transfer in Photosynthetic Antenna Structures
Knox, R.S. **Grant 95-37306-2014**

University of Rochester
Department of Physics and Astronomy **$100,000**
Rochester, NY 14627-0171 **2 Years**

Over half the chlorophyll on earth is bound into light-harvesting protein complexes that funnel captured solar energy into reaction centers where photochemistry begins the energy conversion process. There are several hundred chlorophyll molecules for each reaction center and each chlorophyll can absorb light. It has been known for nearly thirty years that these chlorophylls are packaged into proteins, roughly twenty to fifty at a time, and recently the complete detail of these structures has been emerging through Xray and crystal diffraction studies. Remarkably, energy absorbed anywhere in the system can find the reaction centers, and most of it does. It is known that a physical mechanism known as resonance transfer can account for the broad features of the energy flow, but the process is not fully understood. The standard theory makes the serious assumption that a molecule transferring energy does so after having immediately cooled to the ambient temperature. We have tested this assumption on the chlorophylls and have found that it is not completely valid. The standard theory also fails to apply to certain intriguing energy-transferring molecular pairs and larger aggregates whose role in energy distribution is not clear. The program's overall goal is thus to develop modifications of the standard theory that will account unequivocally for the structure-function relationships of photosynthesis at the earliest stages after absorption of solar energy. The physical and biological events at the initiation of the food chain are arguably one of the most essential molecular processes related to agriculture.

9701965 Gordon Research Conference on "Photosynthesis: Biophysical Aspects''.
Okamura, M.Y. **Grant 97-35306-4398**

Gordon Research Conference **Strengthening Award**
c/o University of Rhode Island **$8,000**
West Kingston, RI 02892-0984 **1 Year**

The goal of photosynthesis research is to answer the question of how sunlight is converted into chemical energy in living systems. Current research has been focussed on structure and function of the molecular complexes that compose the photosynthetic machinery located in cell membranes of plants and photosyntheric bacteria. Major advances have been made in recent years with biophysical approaches to photosynthesis. The Gordon Conference on "Photosynthesis:Biophysical Aspects" brings together leading researchers from diverse fields; structural biology, biophysics. spectroscopy, molecular biology, synthetc chemistry, and computational biophysics to exchange ideas and report recent fndings. Some of the research to be reported include the following: molecular structures of protein complexes determined using x ray diffraction and spectroscopic techniques, the temporal sequence of events resolved using pulsed laser experiments, the simulation of molecular models for energy conversion

processes using new computational methods. The results of this conference will advance our understanding of photosynthesis which forms the basis for crop productivity.

9701960 Mutational Analysis of Overproduced Rieske Fe-S Proteins of Photosynthesis
Kallas, T. **Grant 97-35306-4556**

University of Wisconsin, Oshkosh
Department of Biology and Microbiology
Oshkosh, WI 54901-8640

$125,000
2 Years

Agriculture and global oxygen-production depend on photosynthetic conversion of light energy catalyzed by protein complexes of plant chloroplasts and cyanobacteria. The Rieske iron-sulfur (Fe-S) protein of the cytochrome b_6f complex performs in a key, rate-limiting step of photosynthesis. We have successfully overproduced native and truncated cyanobacterial Rieske proteins to which the characteristic 2Fe-2S cluster can be restored in vitro (in test tubes) or in vivo (within cells). These will be used in mutant analysis overproduction will be used together with electron paramagnetic resonance (EPR), circular dichroism (CD), magnetic resonance (NMR), and fluorescence characterization of protein features required for assembly, catalysis, and intersubunit interactions. 3) Determination by X-ray crystallography or NMR of the structure of mutant proteins with interesting properties. Some studies will be performed collaboratively. The research will contribute to elucidation of structure and function relationships in the Rieske 2Fe-2S protein. Such knowledge will be essential for full understanding of photosynthetic mechanisms and their eventual manipulation for agricultural purposes.

NITROGEN FIXATION/NITROGEN METABOLISM

Panel Manager - Dr. Robert P. Hausinger , Michigan State University
Program Director - Dr. H. Jane Smith

Grants in this program support research which will improve our understanding of how nitrogen is fixed biologically, metabolized and cycled by crop plants. These goals are of importance to sustainable plant production, since availability of nitrogen is a common limiting factor in plant growth, and because of costs associated with application of nitrogen fertilizers. Areas supported by the program include, but are not limited to: chemistry of biological nitrogen fixation; factors limiting biological nitrogen fixation; plant-microbe interactions involved in establishing and maintaining the biological-nitrogen fixation symbioses; and metabolism of nitrogenous compounds by higher plants.

9703455 Genes in *Azotobacter vinelandii* Involved in Cellular Responses to Fixed Nitrogen
Kennedy, C.K. **Grant 95-37305-2067**

University of Arizona
Department of Plant Pathology **$110,000**
Tucson, AZ 85721 **2 Years**

Biological nitrogen fixation is important in agriculture because it provides a source of fixed nitrogen for growth of plants that is non-polluting and not dependent on fossil fuels for production. The enzyme that converts dinitrogen gas to ammonium, nitrogenase, requires the products of approximately 20 *nif* genes for its synthesis and activity. The free-living soil bacterium *Azotobacter vinelandii* is a model organism for the study of molecular mechanisms that regulate the expression of *nif* genes because it is amenable to genetic manipulation and a wealth of knowledge is available concerning *nif* genes and nitrogenase biochemistry and physiology. The *nif* genes are not expressed if sufficient fixed N is available in the environment. In *A. vinelandii* this regulation involves an activator of *nif* gene transcription, NifA, and another protein, NifL, that inhibits NifA activity if plentiful fixed N is available. NifL is therefore a sensor for the fixed N status and previous work from the PI's laboratory demonstrated the involvement of the *glnD* gene product in the conversion of the inhibitory form of NifL to the non-inhibitory form. Whether or not the *glnB* gene product, the PII protein, mediates the effect of GlnD on NifL is to be determined. If PII is not involved then other cellular proteins that interact with GlnD and/or NifL will be sought by isolation of proteins that bind to one or both. Alternatively, mutagenesis followed by selection and screening for mutant strains with relevant regulatory phenotypes will be carried out. Understanding how fixed N regulates nitrogenase availability is necessary for devising strategies to increase the amount of ammonium supplied by nitrogen fixing bacteria to plant partners. This will result in increased yield of food with decreased chemical inputs.

9703547 Physiological Role of A Nod Factor Binding Protein
Etzler, M.E. **Grant 97-35305-4630**

University of California, Davis
Section of Molecular and Cellular Biology **$150,000**
Davis, CA 95616-8535 **2 Years**

The nitrogen fixing rhizobium-legume symbiosis is one of the most important symbiotic associations that occur in nature, since it enables the plants to grow in nitrate deficient soil and results in the replenishment of the soil with nitrogen reserves. The establishment of this symbiosis is dependent on a specific recognition that occurs between the roots of the plant and Nod factors produced by the rhizobial strain that nodulates it. This proposal is based on the discovery of a protein in the roots of the legume, *Dolichos biflorus*, that binds to Nod factors produced by the rhizobia that symbiose with this plant. This research will seek to establish the role of this protein in the plant, particularly with respect to its possible function in the initial stages of rhizobial symbiosis. One portion of this study will be devoted to blocking the expression of this protein in a legume and studying the effects on plant development and rhizobial symbiosis. A second portion of the study will determine whether transfer of the DNA encoding this protein to another legume will alter the host/strain specificity of that legume. The information obtained in this study should enhance our understanding of the molecular mechanisms involved in this important symbiosis and may ultimately have important ramifications on United States agriculture and the world food supply.

9703454 Coevolution of *Frankia* and Actinorhizal Plants
Benson, D.R. **Grant 97-35305-4631**

University of Connecticut, Storrs
Department of Molecular and Cell Biology
Storrs, CT 06269-3044

$90,000
2 Years

Bacteria known as *Frankia* infect and form root nodules on plants that grow in relatively extreme enviroments. They enable their plant hosts to consume nitrogen from the atmosphere (nitrogen fixation) and grow in nutrient poor soils. The diversity of the plants suggests that this bacterial-plant association has appeared sporadically during the evolution of land plants. In order to gauge whether symbioses might eventually be established with related plants we have been studying the degree to which the infecting bacteria have co-evolved with their plant hosts. Comparing the evolutionary trees of the plant and bacterium reconstructs the evolution of this important symbiosis. The relationships of *Frankia* strains with each other, and of the plants with each other, are being established using direct DNA sequencing of bacterial and plant genes purified from root nodules. The information obtained will also be useful for addressing ecological questions about host specificity, geographical distribution of the bacterium and issues of diversity and competition of bacteria in soil.

9703357 Nitrogen Assimilation: Molecular and Genetic Aspects
Barber, M.J.; Solomonson, L.P.; Cannons, A.C. **Grant 97-35305-4381**

University of South Florida, Tampa
Department of Biochemistry and Molecular Biology
Tampa, FL 33612

$5,000
1 Year

Nitrogen assimilation represents one of the fundamental processes of plant metabolism. Inorganic nitrogen is converted to a biologically useful form via two pathways, nitrate assimilation and nitrogen fixation, that require the enzymes, nitrate reductase and nitrite reductase and nitrogenase, respectively. The 4th International Conference on Nitrogen Assimilation: Molecular and Genetic Aspects is designed to bring together researchers in both fields of nitrogen assimilation to share and discuss the most recent advances in these fields and to encourage future collaborations. Topics to be covered in the various conference sessions include the structure and function of nitrogenase, biosynthesis of the various forms of the molybdenum cofactor, uptake and transport processes that support nitrogen assimilation, structure and function of nitrate reductase, light regulation, transcriptional regulation, molecular evolution of nitrate reductase genes and biotechnology. The conference format will include invited presentations by internationally-recognized authorities in the various aspects of nitrogen assimilation and contributed poster presentations.

9703364 Single-Crystal and Solution ENDOR Studies of Nitrogenase
Hoffman, B.M. **Grant 97-35305-4679**

Northwestern University
Department of Chemistry
Evanston, IL 60208-3113

$110,000
2 Years

The biological production of ammonia from nitrogen of the air is achieved in certain microorganisms by the nitrogenase enzyme, and is of critical importance in the agriculture of legumes as well as other crops. However, this reaction continues to defy our understanding, much less any efforts at its modification, or at achieving a useful chemical alternative. A component of nitrogenase called the MoFe protein incorporates the sites of ammonia formation, and we are studying it with a kind of 'structural microscope' with the aim of identifying the atomic features that control the exquisitely tuned catalytic mechanism. The aim is to help to explain the underlying chemical events by providing microscopic information about the composition, structure, and bonding of key intermediates in the chemical process. The techniques we employ combine nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) and are called electron-nuclear double resonance (ENDOR) and electron spin-echo envelope modulation(ESEEN). In favorable cases, like nitrogenase, these techniques offer the prospect of characterizing every atom involved in the reaction at a catalytic active site.

9703356 Mechanism of Nitrogenase Protection by Shethna Protein
Maier, R.J. **Grant 97-35305-4923**

Johns Hopkins University
Department of Biology
Baltimore, MD 21218-2685

$115,000
2 Years

Nitrogen is required for all life forms to metabolize, grow, and reproduce. The biological process responsible for a positive input of N on earth is nitrogen fixation, carried out by useful bacteria, typically in diverse types of soil environments. The nitrogen fixation process carried out by aerobic (O_2-requiring) respiratory bacteria must include physiological mechanisms to protect the

N_2-fixing catalytic units (the nitrogenase enzyme components) from oxygen-mediated damage. The common soil bacterium *Azotobacter* "protects" nitrogenase by use of a protein termed the "Shethna protein' that binds to nitrogenase in high O_2 conditions, rendering nitrogenase resistant to oxygen inactivation. This protection allows the beneficial enzyme to continue to function under adverse high O_2 conditions. The precise protein-protein associations between nitrogenase and the Shethna protein will be determined, and the roles of the Shethna protein in preventing O_2-mediated damage to the molecules making up the cellular machinery of N_2 -fixing bacteria will be studied. Determining the roles of the Shethna protein in preventing toxic oxygen radical production, and the specific sites of contact between the Shethna protein and the nitrogenase will be important for our knowledge to eventually improve upon the stability and efficiency of N_2-fixing systems in general.

9703485 Characterization and Regulation of *noeD*, a Negatively-Acting, Genotype-Specific Nodulation Gene from *B. japonicum*
Sadowsky, M.J; Vance, C.P. **Grant 97-35305-4743**

University of Minnesota, St. Paul
Department of Soil, Water, and Climate **$95,000**
St. Paul, MN 55108 **2 Years**

Specific soil bacteria called *Bradyrhizobium* form an intimate symbiotic association with soybean plants. The symbiosis takes place in special structures on the soybean root called nodules, which contain the bacteria. The bacteria, upon entering into the symbiotic state, gain the ability to convert atmospheric nitrogen gas, N_2, into ammonia, a process called nitrogen-fixation. The bacteria in turn pass this fixed nitrogen to the soybean plant and allow the plant to grow without added fertilizer. Recent nitrogen fixation research has emphasized the use of genetic engineering for the construction of rhizobial strains with enhanced nitrogen fixation. However, due to competition by other indigenous soil bacteria, establishing these superior strains in nodules of field-grown soybean has been, and remains, a critical problem. We have been developing and using a system in which genetic factors present in soybean and *Bradyrhizobium* interact to control competition by indigenous *Bradyrhizobium* population. We have identified several soybean varieties which inhibit nodulation by competitive, but poor nitrogen-fixing bacteria, yet allow nodulation by highly effective N_2-fixing bacteria *japonicum* strains. Recently, we have identified genes in the bacteria and soybean which prevent nodulation by the undesirable *bradyrhizobia*. Our overall project goal is to increase our basic understanding of the genetic contribution of both symbiotic partners and our ability to manipulate them to influence competition for nodulation. This will be done by examining how the host and bacterial genes interact to control competition for nodulation. The experimental plan focuses on integrating research efforts from plant genetics, plant physiology, and microbial molecular genetics.

9703345 Regulation of Nitrogen Assimilation in Alfalfa Root Nodules
Vance, C.P.; Gantt, J.S.; Temple, S.J. **Grant 97-35305-4628**

USDA Agricultural Research Service
Plant Science Research Unit **$115,000**
St. Paul, MN 55108-6024 **2 Years**

Legume plants in symbiosis with rhizobium bacteria obtain most of their nitrogen fertilizer from the air through a process called symbiotic nitrogen fixation. This process occurs in small wart-like growths, termed nodules, which grow on roots. The rhizobium bacteria in the nodules convert atmospheric nitrogen gas to ammonia. Plant enzymes (catalysts) in nodules change the ammonia to amino acids which can be used for growth and seed formation. This process saves the farm economy about $5 billion annually in chemical fertilizer cost. In addition, the use of legumes in farming systems further contributes to sustainability by reducing the potential for nitrogen leaching into ground water and streams. Recently we have isolated three plant genes encoding enzyme critical to root nodule amino acid metabolism: asparagine synthetase (AS), aspartate aminotransferase (AAT), and malate dehydrogenase (MDH). Research funded by this award will identify biochemical and molecular components that regulate the expression of these genes during nodule development. Moreover, using biotechnology and gene transfer approaches, the expression of the genes will be increased with the ultimate goal of improving the efficiency of nitrogen fixation and assimilation.

9703365 16th North American Symbiotic Nitrogen Fixation Conference
Vance, C.P.; Graham, P.H. **Grant 97-35305-4401**

USDA Agricultural Research Service
Plant Science Research Unit **$4,000**
St. Paul, MN 55108-6024 **1 Year**

Legume plants in symbiosis with the soil bacteria rhizobium obtain most of their nitrogen fertilizer from the air through a process called symbiotic nitrogen fixation. This process occurs in small wart-like growths, termed nodules, which grow on plant roots. The rhizobium bacteria in the nodules convert atmospheric nitrogen gas into ammonia. Plant enzymes (protein catalysts) in nodules change the ammonia into amino acids. Through this process 90 million metric tons of nitrogen fertilizer are acquired each year. To replace this nitrogen with industrially produced fertilizer would cost approximately $30 billion annually. Symbiotic

nitrogen is not only cost effective but also is important for sustainable agriculture. It reduces our need for consuming nonrenewable resources and the nitrogen gained by this process is less likely to leach through soil and contaminate ground water. Thus, improvements in nitrogen fixation are important goals for American agriculture. Funds awarded in this travel grant will be used to support U.S. scientists and students attending the 16th North American Symbiotic Nitrogen Fixation Meeting. The objective of this professional meeting is to bring together the North American scientific community working on symbiotic nitrogen fixation to discuss the most recent advances in research and identify approaches to apply this new knowledge.

9703453 Regulation of Arginase in Seed Physiology
Polacco, J.C. **Grant 97-35305-4629**

University of Missouri,Columbia
Biochemistry Department **$95,000**
Columbia, MO 65211 **2 Years**

Soybean is an important national product for domestic consumption and export. Soybean seeds are an important protein source in animal and human nutrition. This protein is deposited in the seed during the green pod stage and is used by the seedling during germination. Proteins consist of 20 amino acids and almost one-fifth of the nitrogen in soy protein is in the amino acid arginine. Animals use the enzyme arginase to break down arginine to produce urea, which they spill daily as excess nitrogen. The developing soybean seed is faced with a dilemma, however. It has high levels of both arginine and arginase, but it does not want the two to interact to divert valuable nitrogen to urea and away from protein. (Animals can move or maneuver to obtain nitrogen, while plants are stuck in one place and are usually growth-limited by available nitrogen.) Our work will address how the developing soybean keeps arginine and arginase separate, and how the two come together during seed germination to provide nitrogen precursors to the seedling. We are testing the hypothesis that arginase is wrapped in a package, away from arginine. During germination, the seedling puts holes or specialized vents in the wrapping, allowing arginine to get in, break down, and the breakdown products to leave. We believe the package is the mitochondrion, the energy cell within the larger cell. This work has implications for increasing protein in soybeans and for understanding how the plant conserves precious nitrogen.

9703754 Nitrogen Fixation in Photosynthetic Bacteria: New Gene Regulatory Mechanisms
Kranz, R.G. **Grant 95-37305-2065**

Washington University
Department of Biology **$130,000**
St. Louis, MO 63130-4899 **2 Years**

Can U.S. farmers ever look forward to increased use of biologically fixed nitrogen, produced by bacteria (i. e., prokaryotes), the only organisms on earth which naturally fix atmospheric nitrogen gas into ammonia? Sixty years ago the health industry asked a conceptually similar question about bacteria: can U.S. citizens (and soldiers) ever look forward to major reductions in deaths and illness due to infectious diseases caused by bacteria (e.g.. pneumonia, tuberculosis)? The answer was yes, but the result required many decades of research in understanding the basis for the disease process and how to overcome infectious bacteria (e.g.. with knowledge of antibiotics and targets). Likewise, if we are to use microorganisms to economically and in an environmentally safe manner produce sources of natural fixed nitrogen we must understand the basic mechanisms by which this process occurs and is regulated. Our previous research supported by the USDA has shown that some photosynthetic bacteria control over 30 genes required for nitrogen fixation by using two new mechanisms of gene regulation not yet described in other bacteria. The first mechanism concerns how specific RNAs are made from DNA only when the RcNtrC protein senses a poor nitrogen environment. Using biochemical and genetic engineering approaches we will further analyze the RcNtrC activator by showing what new type of RNA polymerase it activates for this unique control. Second, study of another mechanism will answer how RcNtrC controls the protein synthesis machinery in the cell to make the NifR3 protein from RNA under nitrogen-poor conditions.

9703457 Molecular Genetics of a Second *nif* Gene Cluster in *Anabaena variabilis*
Thiel, T. **Grant 97-35305-4970**

University of Missouri, St. Louis
Department of Biology **$110,000**
St. Louis, MO 63121-4499 **2 Years**

All plants need nitrogen, which may be provided chemically as fertilizer or biologically from bacteria or blue-green algae (also called cyanobacteria) in soil and water. Biological nitrogen fixation depends on an enzyme called nitrogenase that converts the nitrogen gas in air to ammonia, a form of nitrogen that plant cells can use to grow. The production of this enzyme is tightly controlled so that cells do not waste their energy making the enzyme when it is not needed. The primary objective of this research project is to use the tools of molecular genetics to understand how the cyanobacterium, *Anabaena variabilis*, controls one set of genes that make this important enzyme, nitrogenase. These genes, called *nif2*, do not produce nitrogenase unless the environment

in the cell can support the action of this enzyme. Understanding how cells control when genes are expressed is important both in basic and in applied science. By understanding how the organism controls the *nif2* genes, which allow the cells to convert nitrogen gas to ammonia, we can better control biological nitrogen fixation which supports the growth of many crops throughout the United States and the rest of the world.

9703361 14-3-3 Proteins and the Regulation of Nitrate Reductase by Phosphorylation
Huber, J.L.; Huber, S.C. **Grant 97-35305-4745**

North Carolina State University
Dept. of Horticultural Science
Raleigh, NC 27695-7609

$140,000
2 Years

Nitrate reductase (NR) is responsible for the first of a series of reactions whereby nitrate is reduced and assimilated into amino acids essential for plant growth and development. The plant uses several mechanisms to control NR, including modifications to the enzyme's structure which result in activity changes. These structural changes are brought about by a process called protein phosphorylation, wherein a phosphate molecule is enzymatically bound to a specific site on NR thereby altering its structure and ability to assimilate nitrate. NR is one of only a small number of proteins known to be controlled by phosphorylation. Although it is clear that it is a major mechanism for the regulation of many processes in the cell, much remains to be learned about even the most general aspects of phosphorylation. Recent results demonstrated that the regulation of NR by phosphorylation involves a binding protein known as a 14-3-3 protein. Originally isolated from brain tissue, the 14-3-3 family of proteins is involved in the control of diverse pathways and processes in both plants and animals. Our work will examine how 14-3-3 proteins, together with phosphorylation of NR, lead to changes in the rate at which plant NR assimilates nitrate. The insights gained from this research will contribute immediately to our understanding of the complex mechanisms of enzyme control in both plants and animals which involve phosphorylation and 14-3-3 proteins. In the longer term, the work may lead to plants with superior capacity to utilize nutrients.

9706584 Heme-based Regulation of Nitrogen Fixation: Structure Function Relationships in FixL
Rodgers, K.R. **Grant 97-35305-5158**

North Dakota State University
Department of Chemistry
Fargo, ND 58105-5515

PECASE* Awardee 1996
$199,466
3 years

FixL is a unique oxygen-sensing protein that regulates the level of nitrogen fixation (production of ammonia from atmospheric nitrogen) by *Rhizobium* bacteria in response to the amount of oxygen present within the cell. This regulation is crucial to the cell because the process of fixing nitrogen can only proceed under conditions of low intracellular oxygen levels. FixL contains the same heme found in mammalian hemoglobin. When oxygen is present at levels that inactivate the cell's nitrogen fixing system, this heme is able to form a chemical bond with the oxygen. The formation of this bond triggers an ill-defined chain of events that switches off the cell's production of its nitrogen-fixing system.

The goal of this project is to elucidate the initial steps in the aforementioned chain of events at the molecular level. This will be accomplished using laser-based methods to probe the oxygen-sensing heme and its protein environment. A detailed understanding of the interplay between the heme and its immediate surroundings is expected to provide a foundation for (a) more extensive study of this signal-transduction system and (b) the rational design of genetically engineered bacteria capable of efficient ammonia production. Such organisms could be applied to agricultural fields to generate available nitrogen. This method of fertilization could potentially replace current methods such as anhydrous ammonia, the production, transportation and application of which is hazardous and energy-intensive.

*Presidential Early Career Awards for Scientists and Engineers

9703482 5-Aminolevulinic Acid (ALA) Formation in Legume Root Nodules.
O'Brian, M.R. **Grant 95-37305-2253**

State University of New York, Buffalo
Department of Biochemistry
Buffalo, NY 14214-3000

$100,000
2 Years

Soybeans and other legumes are extremely important to agriculture in the United States and throughout the world. These plants are able to grow under conditions in which other crops such as corn and wheat cannot because legumes establish a relationship with beneficial bacteria which provide them the essential nutrient nitrogen in a usable form. This group of bacteria, collectively called *Rhizobium*, resides within nodules on the legume root, and converts nitrogen in the air into ammonia by a process called nitrogen fixation. In our view, nitrogen fixation must be a central feature of sustainable agriculture strategies. Thus, we must understand the molecular basis of these interactions toward the goals of maximizing the benefit to the legume, and

extending the process to other important crops by genetic engineering. Nitrogen fixation is energy-demanding for both the legume host and the rhizobia bacteria, and this project seeks to understand crucial aspects of the energy utilization system from the plant perspective in the soybean-*Bradyrhizobium japonicum* symbiosis. The compound 5-aminolevulinic acid (ALA) is the basic building block of heme, a molecule necessary for respiration in all aerobic organisms. Whereas the amount of heme present in most plant tissues is low, it is extremely high in root nodules as part of a plant hemoglobin necessary for nitrogen fixation. Accordingly, ALA synthesis must increase dramatically during nodule formation, and we are working to understand how this induction occurs. We have isolated the two committed soybean genes for ALA synthesis, and have found that they are highly regulated by different mechanisms. We are identifying factors that mediate this control.

9703349 Assembly of the Iron-Protein of Nitrogenase (Is the NifM protein peptidyl-prolyl *cis/trans* isomerase?)
Gavini, N. **Grant 97-35305-4741**

Bowling Green State University **Strengthening Award**
Department of Biological Sciences **$90,000**
Bowling Green, OH 43403-0020 **2 Years**

The air is composed of 79% nitrogen gas (N_2), however, N_2 must be converted to a fixed form before it can be used to synthesize proteins, nucleic acids and other cellular components. Therefore, nitrogen fixation is of fundamental importance in the biosphere. The N_2-fixing bacteria have an enzyme called nitrogenase encoded by *nifHDK* genes. Besides these structural genes that encode nitrogenase, there are a number of *nif*-specific genes and their protein products. Although the functions of many of these genes have not been determined, the genes have been shown to be necessary for the maturation, assembly and reactivity of nitrogenase. We compared the *nifM* genes and generated a consensus sequence which was then compared to the conceptually translated nucleotide sequence data bases. This comparison showed that the carboxyl terminal region of the NifM-proteins shares significant homology with the family of proteins called peptidyl-prolyl *cis/trans* isomerases. As a first step to identify the functional properties of the *nifM* product, we propose to purify the NifM-protein and to test its ability to f unction as a peptidyl-prolyl *cis/trans* isomerase. Since the levels of the *nifM g*ene expressed from its native promoters are very low, we need to construct a strain that can overexpress the NifM-protein. The specific goals of the research in my laboratory during this grant period are as following: 1. To construct strains that can overexpress functional NifM protein, 2. To purify the NifM protein to homogeneity, 3. To test its ability to function as a peptidyl-prolyl *cis/trans* isomerase.

9703546 Crystal Structure of DctD
Nixon, B.T.; Farber, G.K. **Grant 97-35305-4742**

Pennsylvania State University, University Park
Department of Biochemistry and Molecular Biology **$95,000**
University Park, PA 16802 **2 Years**

All organisms must detect changes in their environments, and respond appropriately. Integrating sensing with responding requires signal transduction, where detection generates a signal that is received by a responding mechanism within the cell. The predominant mechanism of bacterial signal transduction is the two-component structure: a sensor protein, and a response regulator protein. Together, the dozens of two-component signal transduction systems encoded in a bacterial genome permit the organism to cause diseases in animals and plants, to direct cell movements, to degrade toxic aromatic compounds, and to make other useful enzymes and metabolic products. Despite the obvious importance of this type of signal transduction, the physical basis for how it works is not known for even a single example. This study focuses on the DctD protein, a two-component response regulator that is essential for the soil bacterium *Rhizobium meliloti* to fix nitrogen in symbiosis with its legume host plant, alfalfa. DctD has three physical parts: a DNA binding domain, a gene activation domain, and a two-component receiver domain. The study will determine the 3-dimensional structure of the two-component receiver domain in two forms: in one form, it causes the gene activation domain to be active; in the other form it causes the gene activation domain to be inactive. Comparing the two structures should suggest a physical mechanism for how the receiver domain communicates with the gene activation domain. This information might then be used to improve crop yields by enhancing nitrogen fixation, or by controlling soil-borne pathogens.

9703548 The Nodulin 26 Symbiosome Aquaporin: Permeabililty Properties and Regulation by Calcium-dependent Kinases.
Roberts, D.M. **Grant 97-35305-4742**

University of Tennessee, Knoxville
Department of Biochemistry, Cellular and Molecular Biology **$188,000**
Knoxville, TN 37996-0840 **3 Years**

Nitrogen fixation symbioses between soil rhizobia bacteria and legumes represent a specialized process in which the bacteria fixes atmospheric nitrogen for the plant host. This process is of fundamental agricultural importance since it: 1. provides a major source of usable nitrogen for legume crops, as well as 2. constitutes a major pathway for assimilation of atmospheric nitrogen into

the global nitrogen cycle. The understanding of the various biochemical and genetic factors that control the formation and maintenance of this symbiosis is an essential first step for the future design of stratagies to improve this process. Our research is focused on a protein from soybean known as nodulin 26. Nodulin 26 is made specifically by the soybean plant during the formation of the nitrogen fixing nodule and is found on the plant membrane which surrounds the rhizobia bacterium. Our past findings suggest that this protein plays an important role in the transport of metabolites and the maintenance of a water balance between the bacterium and the plant which is essential for optimal nitrogen fixation. The research in this project addresses the following questions: 1. What is the range of metabolites transported through nodulin 26 and how do they affect nitrogen fixation? 2. What molecular and genetic strategies does the soybean cell use to control transport through nodulin 26? 3. How is nodulin 26 transport regulated in response to changing environmental conditions (e.g., drought and carbon limitation) that adversely affect nitrogen fixation? 4. what are the identity and function of regulatory proteins (protein kinases) that control nodulin 26 function? This work will provide a clearer picture of the role of nodulin 26 in communication between the bacterium and the soybean host.

9704014 Genetic and Molecular Analysis of Early Nodulin Gene Regulation in *M. truncatula.*
Cook, D.R. **Grant 97-35305-4821**

Texas A&M University, College Station
Department of Plant Pathology and Microbiology
College Station, TX 77843-2132
$175,000
3 Years

The rhizobium-legume symbiosis constitutes one of the major sources of reduced nitrogen in the global nitrogen cycle. Historically, crop rotations with legumes have been an important mechanism for increasing agricultural productivity, while in modern agriculture high yields are typically associated with the input of industrially-derived nitrogen fertilizers. This practice accounts for one of the primary expenses of modern agriculture, and is an important source of agricultural ground water contamination. Improving the efficiency of symbiotic nitrogen fixation could reduce the dependence on nitrogen fertilizers and may have important implications for sustainable agriculture. However, improvement of symbiotic nitrogen fixation through biotechnology will rely heavily on understanding the plant mechanisms that control symbiotic development, and on the identification of the responsible plant genes. The long term goal of this research is to understand the plant molecular-genetic mechanisms that control symbiotic development in the legume *Medicago truncatula.* Towards this end we are investigating plant gene regulation at the onset of symbiotic development. Specifically, our goal is to identify the mechanisms that underlie early changes in host gene expression. Such knowledge is expected to provide insight into mechanisms for control of symbiotic development, and may contribute to the long-term goal of improved nitrogen fixation.

9703353 Biochemical Characterization of Nitrogen-Responsive Lipoxygenases
Grimes, H.D. **Grant 97-35305-4924**

Washington State University, Pullman
Department of Botany & Genetics and Cell Biology
Pullman, WA 99164-4238
$100,000
2 years

Nitrogen is an element required for all life forms to exist. Plants, especially legumes like soybeans, play a critical role in thenet positive input of nitrogen into life on this planet by assimilating this nutrient from the soil by various mechanisms. Plant growth, development, and productivity are heavily dependent on the processes that accumulate nitrogen from the soil and distribute this essential nutrient into the plant tissues, like seeds, that humans consume. Thus it becomes critical to understand the processes that are involved in metabolizing nitrogen and in distributing this nutrient throughout the plant. Our research focuses on specific proteins, termed lipoxygenases, present in the leaves of soybean which are tightly regulated by the available nitrogen and may function to store this nitrogen until it can be subsequently redistributed to the seed. There are several genes for lipoxygenases in soybeans that are expressed at different stages of development and in different parts of the plant. Specific lipoxygenases appear to be associated with a unique cell layer that controls the movement of many essential compounds, including carbon and nitrogen, throughout the plant. This research will investigate the biochemical properties of these lipoxygenases to determine their role in nitrogen movement, metabolism, and distribution. In the future, manipulation of these processes may lead to plants able to assimilate and distribute nitrogen more efficiently.

9703351 Travel by NRA-Supported Scientists to the 11th International Nitrogen Fixation Congress

Ludden, P.W. **Grant 97-35305-4396**

University of Wisconsin, Madison
Department of Biochemistry
Madison, WI 53706-1569
$5,000
6 Months

Funds in this award will be used to support the travel of USDA/NRI-supported scientists to attend the International Congress on Nitrogen Fixation to be held in Paris, France July 20-25, 1997. This meeting is the most important meeting of scientists in the

area of nitrogen fixation, and all aspects of nitrogen fixation are covered at this international symposium series which occurs every second or third year. The participation of US scientists is critical to the success of these international symposia, and it is important to the scientific effort in the United States that NRI-supported scientists attend this symposium where the latest information in crucial areas of nitrogen fixation will be presented. A special effort will be made to support the travel of young scientists, women and minorities for attendance at this meeting.

9703456 Steenbock Symposium on the Biosynthesis and Function of Metal Clusters in Enzymes
Roberts, G.P. **Grant 97-35305-4397**

University of Wisconsin,Madison
Department of Bacteriology; Center for the Study of Nitrogen Fixation
Madison, WI 53706-1521

$3,000
6 Months

Funds from this award will be used to support the travel of speakers to attend the 1997 Steenbock Symposium to be held in Madison, Wisconsin June 10-14, 1997. The topic of this symposium will be the biosynthesis and function of metal clusters for enzymes. Within this topic of the symposium are a number of areas of central interest to NRI/USDA-funded projects. These include nitrogen fixation, nitrate reduction, denitrification, enzymes involved in nitrogen and C1 metabolism, and other aspects of metal metabolism involved in critical assimilatory pathways important to agriculture. An effort will be made to use these funds to support the travel of young scientists, women and minorities to attend the Steenbock Symposium. This symposium is a unique effort to bring together scientists working on various aspects of primary assimilatory pathways important to agriculture to discuss common features among pathways of biosynthesis of complex metal clusters involved in those different pathways.